

Jewels in the Mist

A synopsis on the highly endangered butterfly species
the Violet Copper, *Lycaena helle*

Jan Christian Habel, Marc Meyer, Thomas Schmitt (eds.)
including a Preface by James Mallet
and a Prologue by Henri Descimon



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Cover-pictures: Typical misty environment and habitat of *Lycaena helle* in the Massif Central, France (Marc Meyer), and a female of the Violet Copper in the Westerwald area, Germany (Klaus Fischer).

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Jan Christian Habel & Thomas Schmitt

Editorial

Jan Christian Habel¹, Marc Meyer² & Thomas Schmitt^{3,4}

¹Terrestrial Ecology Research Group, Department of Ecology and Ecosystem Management, Technische Universität München, D-85350 Freising-Weihenstephan, Germany

²D-66706 Kesslingen, Germany

³Department of Biogeography, Trier University, D-54286 Trier, Germany

⁴Senckenberg German Entomological Institute, D-15374 Müncheberg, Germany

The Violet Copper *Lycaena helle*, a blue-glimmering tiny jewel inhabiting misty, cool and moist habitats with its food-plant *Polygonum bistorta*, is one of the first butterflies flying in the first sunny days of spring in Europe. This little and highly endangered butterfly has fascinated many scientists over the years. To understand its habitat requirements, larval ecology and behavioural ecology, population structures, biogeography and to evaluate its recent status, manifold studies were elaborated to understand and conserve this butterfly species of the cold past. This creature is both, optically and biologically exceptional: The large morphological variability fascinated lepidopterologists, and the species was split into nine subspecies by the year 1982. These subspecies occur mostly restricted to single mountain archipelagos, and the highly isolated mountain populations of the Middle Mountains of Central Europe provide excellent prerequisites to test for effects as environmental stress and the ecology and biology of relict populations.

First scientific analyses on the population structure of the Violet Copper in highly fragmented habitats were conducted in the Westerwald, western Germany, in 1999 to analyse the impact of habitat isolation, habitat size and habitat characteristics on the occurrence of the butterfly. In 2008, species-specific primers for detecting polymorphic microsatellites were developed and applied to analyse population genetic structures over various temporal and spatial scales – including a conservation genetic focus as well as a biogeographical view (based on populations from the Polar Circle to the Pyrenees across the entire western Palaearctic distribution range of *L. helle*). Further studies on habitat demands and larval ecology were analysed to delineate the seemingly identical ecological niches of the syntopically occurring species *Procllossiana eunomia* and *L. helle*. Finally, the distribution of the climate niche of *L. helle* was modelled to picture its palaeo-distribution and to derive future climate (and land-use) change scenarios and its impact on the butterfly's occurrence: the resulting prognoses are not optimistic. Today, the Violet Copper is listed on the appendix II and IV of the Habitats directive and is included in several red lists, but still declining over Central Europe.

For this book, we invited scientists who worked and still are working on this fascinating organism to contribute their knowledge and results from the fields of behavioural ecology, population biology, conservation biology and population genetics (including phylogeography and conservation genetics), as well as climate and land-use modelling - puzzling together a complete synopsis on this *Jewel in the mist*. However, these valuable details on this taxon shall not provide a simple synopsis on this taxon, but evidence the necessity to combine various methods and to collaborate in large scientific and conservation networks to understand and cope with the recent biodiversity collapse.

December 2013

Jan Christian Habel, Marc Meyer, and Thomas Schmitt

Preface

James Mallet

Biology Laboratories, 16 Divinity Avenue, Harvard University, Cambridge, MA 02138, USA,
and Department of Genetics, Evolution and Environment, Gower St., London WC1E 6BT,
England, E-Mail: jmallet@oeb.harvard.edu

“It is interesting to contemplate an entangled bank, clothed with many plants of many kinds, with birds singing on the bushes, with various insects flitting about, and with worms crawling through the damp earth, and to reflect that these elaborately constructed forms, so different from each other, and dependent upon each other in so complex a manner, have all been produced by laws acting around us.”

Charles Darwin (1859) “On the Origin of Species,” p. 489.

Butterflies are perhaps the most appealing of invertebrates to amateurs. They’re not spiky like ants and praying mantids, or hard-looking like beetles, or slimy like slugs and snails, or even wriggly like worms (except for the caterpillars, of course). And they’re beautiful, and fly mostly in sunny, warm weather when human spirits are raised.

Butterflies are also diverse, having maybe as many as three times the numbers of species globally that birds have. Butterflies represent about a tenth of the ~170,000 described species of Lepidoptera, which themselves form about 10% of the ~1.9 million described species of living organisms. Thus, butterflies represent around 1% of the diversity of known life on our planet.

For both these reasons, butterflies have become extremely important in conservation. In my native Britain, Butterfly Conservation is the second biggest conservation-oriented society, after the Royal Society for the Preservation of Birds. In Britain also, butterflies have shown rather strong declines in population size (Thomas et al. 2004), particularly because many of them are attached to increasingly fragmented and endangered habitat types such as chalk grasslands or scrubby woodlands. While the situation in Britain is particularly dire, the same problems exist, though perhaps at a somewhat earlier stage of destruction, across Europe. Both butterflies and birds are important flagships of conservation. Today we are increasingly concerned with conservation, not just of endangered species, but of the entire diversity of the ecosystems to which these flagship species belong.

People have been in Europe for a long time, and an astonishingly high proportion of butterflies seem to be addicted to habitats that have been traditionally altered by humans. For example, grasslands grazed by sheep seem important for many butterflies, including the flagship species, the Large Blue *Maculinea* [*Phengaris*] *arion*, which after many years of decline finally went extinct probably as a result of our ignorance of its biology. The caterpillar of this species is an obligate nest parasite of a particular *Myrmica* ant species. In late instars, it is taken into the nest by the ant workers, where it survives by eating ant larvae. This particular ant can only survive in our chilly climate on the sunniest and most highly grazed grasslands. Conservationists thought that by buying land with the remaining populations of the Large Blue from farmers, they would save the species. Unfortunately, the link with sheep-grazing was not known at the time, and the loss of the farmers' sheep led to the final extinction. Today, the link is understood, largely due to the detailed ecological studies of Jeremy Thomas performed during the final gasp of the species' survival in England (Thomas 1980). Since then, Large Blues have been reintroduced from mainland Europe into the original English range, and appropriate grazing pressure applied. The new butterfly population is now spreading across its former range. The Large Blue work shows how detailed ecological and biological study can enable a conservation success story.

While this conservation success is of course mainly about a single, endangered species at the edge of its range, the conservation effort for the Large Blue has many ecosystem-scale conservation benefits. Many, perhaps most plants specific to chalk downlands also thrive only in well-grazed sites. Suites of other butterfly species also show increases in population size on land managed specifically for the Large Blue across Europe. For example, the Pearl-bordered Fritillaries (*Boloria euphrosyne*) and other violet-feeding butterflies do better on land under Large Blue conservation management. The link is again through the ants, but this time involves plants as well. Ants collect violet seeds, which have special oily parts (elaiosomes) that the ants find nutritious. From the plant's point of view, this is advantageous because it leads to seed dispersal by the ants. The seeds tend to germinate best in abandoned ants nests, and the Large Blue parasitism can cause greater levels of ant nest desertion, so speeding germination of more violets. The Pearl-bordered Fritillary also prefers to lay on violets growing on deserted ants' nests, and so these various associations lead to a higher population of the butterflies (Spitzer et al. 2009).

This is just one example of the extraordinary links between species typical of Darwin's "entangled bank". Darwin wrote "On the Origin of Species" when he was living in the village of Downe just outside London. Every day, he would walk around a particular route ("the sand walk") through a wood lot he planted beyond the end of his garden. It might not be an accident that his wooded walkway overlooked a diverse chalk hillside, patches of which are still preserved, and to which a few iconic chalk downland species still cling. Today, however, the majority of the grasslands at Downe have been converted to fields. The only preserved patches now form part of a golf course.

Conservation in the Large Blue is perhaps the best known example where knowledge of butterfly biology is useful. But in many other species, much less is known about habitat requirements. However, it is known that conservation of traditionally grazed chalk downlands aids the preservation of many of our rarest species. In general, conservation of sites containing rare species will tend to enhance the diversity of microhabitats that other species, both common and rare, inhabit. In butterflies, it generally tends to be true that sites with high diversity of one butterfly group tend also to have high diversity of other butterfly groups (Beccaloni and Gaston 1994). There are, of course, limits to the generalization that butterflies form a useful “indicator” group for conservation value. For example, high bird diversity does not necessarily indicate high butterfly diversity or vice-versa (Prendergast et al. 1993). Just as we need to study the biology of individual species to understand their specific ecological requirements for conservation, we will also have to study in great detail the many ecological links between species to understand the conservation of biodiversity in general.

In Europe as a whole, our understanding of butterfly ecology and butterfly conservation is perhaps the strongest in the world. The current volume, a monograph on a particularly attractive European butterfly Violet Copper *Lycaena helle*, is going to be very useful in this regard. The butterfly exists in scattered populations across its range, many of which are under threat. The detailed studies collected in this volume will inform conservation efforts for this species, and contribute to the understanding of the biodiversity of Europe in general. The studies presented here of this beautiful little species will help us preserve for our descendants a few of those tangled banks that were inspirations to our ancestors as well as ourselves.

Harvard University, November 2013

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Prologue

A lifetime with the Violet Copper *Lycaena helle*

Henri A. Descimon

Henri Descimon, honorary Professor, Université de Provence, Marseilles. Personal address: 2
Boulevard Rougemont F-13012 Marseille, France, E-Mail: Descimon.henri@free.fr

‘The vessel of Science follows its glorious way well above the ordinary vicissitudes of human life’. This is the generally admitted conception, but the most deeply entrenched so that it remains implicit. An extreme version of this idea has been presented by Imre Lakatos (1978), who argued that scientific research follows its own way, like a computer program, which should use scientists as Turing machines, without respect to their human nature. However, doubt has been cast about this almost religious (or even inhuman) idea of science. In particular, the ‘Dadaist’ philosopher Paul Feyerabend (1975) considered that fantasy and ‘anything goes’ mental processes are of paramount importance in scientific discovery. Likewise, the sociologists Bruno Latour and Steve Woolgar (1979), who immersed themselves in the research laboratory led by the Nobel Prize winner Roger Guillemin at Salk Institute to study the way in which scientific facts are produced, observed that research is an activity where human beings behave in the same way as in any other one. And those who still consider that ‘Method’ (in the Cartesian meaning) is working alone in the progress of science could acknowledge the narrative of the discovery of PCR by Mullis (1993).

My aim here will be to describe in an autobiographic perspective the relations between the real life of a commonplace scientist and his commonplace kind of research, namely ‘the biology (*sensu lato*) of a little butterfly, *Lycaena helle*, the Violet Copper’. That way, I will be perhaps able to bring a tiny bit of light upon ‘the production of scientific facts’. However, I must specify that this Copper has never been my main research material, but rather a well-beloved Cinderella to which I devoted intense but scanty moments of my life.

DISCOVERY

It was in May 1958. Since the beginning of the 1950s, I felt a passion for butterflies. In 1957, I had presented a dissertation on the biology and ecology of central Pyrenean butterflies under the direction of Professor P-P Grassé. I had already in mind to devote

my life to Lepidopterology. In the year 1958, I was a candidate to the 'Agrégation de Sciences Naturelles', a competitive examination, and quite a challenging heat (and a speciality of the French educational system). After the written tests, there was a lag before the oral ones. I was overexerted and at the limit of collapse. My attentive parents offered me a one week trip, for regaining some mental strength; I chose the Oriental Pyrenees as a target, since I expected to find a large butterfly diversity there.

Amongst the various species I hoped to observe and capture, there was one, almost mythic, which had been recorded from this region: *Lycaena belle*. However, Porté, the initial locality where it had been discovered by Deslandes in 1930 (Deslandes 1930), was located at the extreme NW tip of the department and out of my reach. Fortunately, a keen and friendly amateur lepidopterist, Auguste Chéneau, had found another, more accessible flight place, the Col de la Quillane, close to Montlouis, and gave me its precise location. From Vernet les Bains, in the lower part of the Tet valley, where I resided, I took, very early in the morning, a winded bus, which climbed slowly along the sinuous and narrow road to Montlouis. As soon as we reached this small Vauban citadel, I rushed along the smaller road to Col de la Quillane and climbed as fast as I could.

Still not too late in the morning, I reached the pass; weather was glorious and the sun was shining upon a landscape, which looked as untamed as at the creation of the world. Still today, I feel the impression that this site is representative of *L. belle*'s biotope *par excellence*. It lays amongst a Mountain Pine (*Pinus uncinata*) clear woodland, sloping down gently towards the North, crossed by some marshy thalwegs full of *Polygonum bistorta*, the butterfly's food plant. But, to convey the tone of this place, one must add the noise of the rills, the freshness of the air and the spring flowers as Marsh Marigolds (*Caltha palustris*), white Buttercups (*Ranunculus aconitifolius*) and dwarf Valerians (*Valerianella*). I will find it again on every time I will meet the Violet Copper.

I readily spotted a brisk, never seen small butterfly of an 'impossible colour' (Crane and Piantanida 1983), the Violet Copper! At that time, my 'naturalist' and 'collector' sides were deeply intricate, and I managed to catch a series - today we should say 'gathered a sample' - of the species. However, I had been already educated to limit my catches to a reasonable size, perhaps not enough to allow serious biometrical analyses - I was far from guessing that such studies had been (Bernardi and de Lesse 1952) and would be further realized (Meyer 1981a,b, 1982). And the day was advancing; I had to rush back to catch the return bus. The small butterflies I collected are still as a treasure in my collection and this day always shines in my memory.

AT THE PURSUIT OF THE VIOLET COPPER

I became a young natural history teacher. Algeria war was still in course and I had to spend a part of my military duties in this country. Actually, I devoted three years of my life to these obligations, but I was privileged, since I spent the first and the last year of this period at the military school of Autun in the southern Morvan, as a teacher. This gave

me the opportunity of thoroughly visiting the Morvan massif and to observe extensive marshy habitats, clad with *Polygonum bistorta*, at an altitude which perhaps could be suitable for *L. helle*. However, even in the very best looking places, *L. helle* (as well as its ecological companion *Proclissiana eunomia*) was absent; I felt a kind of frustration and even found that this absence was abnormal or even 'scandalous'!

During the same time, I also visited flight places of *L. helle*, such as the neighbourhood of the Bellefontaine lake in the Jura and the Monts de la Madeleine. I must confess that I retain only a vague remembrance of these trips, probably because I did not meet with any illuminating observations at these opportunities. Actually, for me, in this period, the Violet Copper represented not much more than any other butterfly species.

Stunningly, it is not my stay in the small town of Autun causing my further interest for *L. helle*, but the short and rather unfortunate part of the military duties I spent in Algeria. There, I caught an amebiasis, which turned out to be rather severe. At the expenses of the army, I was granted to spend a cure period during the 1960s at Châtel-Guyon in the northern Massif Central, where thermal water was reputed to be efficient against this disease. By coincidence, I then worked as an assistant at Ecole Normale Supérieure, where I taught the students for the Agrégation de Sciences Naturelles; I was therefore free in May, as when I prepared this examination in 1958. And what could I do in the moments of freedom let by the cure schedule?

Châtel-Guyon was close to the Sancy massif, where *L. helle* was known to be present. Of course, the most obvious goal I could fix to myself was discovering new localities of *L. helle* in this region. It is at that time that my interest for the Violet Copper moved from collection towards a more scientific horizon, which we could name, say, ecogeography. That way, I began to define guidelines for searching systematically favourable habitats of *L. helle*. However, my scientific ambitions in this field were limited since my main research subject was quite different: the biochemistry of pteridines. One must keep in mind that, at that time and in France, laboratory work was an obliged passage in a scientific carrier, exclusive of other activities; in present days, an interface between field and laboratory is widely developed by molecular techniques.

The Violet Copper was thus granted only nibbles of my time. The research tools were not as prestigious as those I used in the laboratory: a good 1/100 000 Institut Géographique National map (with contour lines), a butterfly net, boots and ... a car! The results I obtained and the publications I made (Descimon 1964, 1965) were quite modest and indeed far below a 'professional' level. However, from the point of view of what I felt, it appeared that discovering a new locality of *L. helle* was as exciting as isolating an unknown pteridine. From 1963 to 1968, I mainly screened the Mont Dore massif and established a rather complete map of the distribution of the Violet Copper in this region.

However, the Massif Central is dissected into many isolated secondary mountain ranges, each of which constituting a unit, geographically and ecologically different from the other ones. It was tempting to search *L. helle* in each of these ranges; actually, this work had been already outlined by P. Réal (1962a,b,c,d), and I continued it on a systematic basis, contriving a 'suitability index', which could both explain and predict the

presence of *L. helle* in a given region (Bachelard and Descimon 1999). As a result of this exploration, I concluded that the butterfly species existed in a minority of regions of the Massif Central, with a major population nucleus located in the Sancy range. South to this region, I found that *L. helle* was also present in the Cézallier and even reached the Cantal massif at Bois de la Piniatelle, but without penetrating inside of it, a surprising lack. Therefore, the gratifying thrill to have discovered 'something new' was superimposed by the itch of an unanswered question. These psychological mechanisms have something of an addiction; they are constitutive of the researcher's mentality.

In the following years, I visited the last regions unknown to me from where the Lycaenid was reported, like a markedly isolated and restricted spot in the Monts de la Madeleine, a NW outlier of the Massif Central (where it had been discovered by Guérin (1959)), and the Ardennes, a little patch of which is located on the French territory. In addition to the Cantal massif, I was intrigued by the absence of records from certain regions where the 'suitability index' predicted the presence of the Violet Copper; the most obvious were the Vosges, but they had been reputedly well explored by several generations of Alsatian entomologists, and *L. helle* was unlikely to be present there.

However, in June 1970, for conscientiously completing my research program rather than for allaying an itching question, I enjoyed a short bit of free time to make a short trip to the Vosges. The previous studies about butterfly fauna had been mainly performed on the eastern side of the watershed, with a prevailing dry continental climate, unfriendly to *L. helle* as my explorations in the Massif Central already taught me. I therefore focused my explorations on the moister Lorraine side. On the beginning, as I expected, the exploration proved to be disappointing, and suggestive biotopes lacking. As a solace, there was a 'great cascade' in the neighbourhood of Tendon village that I decided to visit. On the way, I glimpsed an elusive, improbable purplish butterfly, as I saw so many ... My spinal cord preceded my brain, and there was indeed an old *L. helle* male in the bottom of my net. So great was my surprise that I questioned myself: on the previous day, I visited the Ardennes populations of the butterfly; could I have overlooked a butterfly quenched in a fold? Actually, I soon found other places where *L. helle* was thriving; the species was thus indigenous to the Vosges (and the 'suitability index' confirmed its predictive value)!

Another principle of the psychology of research was also verified: the more unexpected a finding, the more exciting. Unfortunately, I had not enough time to thoroughly explore the region and had to leave the Vosges before gathering exhaustive information about the distribution of this butterfly species. In place of publishing a provisional record of my discovery, I mentioned it incidentally in an article about another subject (cunningly, I believed!) (Descimon 1976). However, this detail did not escape the attention of Jean-Claude Weiss, who took advantage of this discreet record; he found the butterfly with no difficulty and described a new subspecies, which he dedicated to a lepidopterist from the Vosges, very estimable indeed, but who had never observed *L. helle* in this region (Weiss 1977)!

The discovery of *L. helle* in the Vosges brought the exploration of France to an end, and I intended to present a complete survey of the distribution of the butterfly in this country. However, at the very moment I was ready to submit this work, Marc Meyer

(1981a,b, 1982) issued a series of papers, which covered the same field and much more. I was outstripped again! This was not a tragedy since this subject was ranked at second order in my scientific activity. I turned my interest towards other targets concerning *L. helle*.

‘CORRECTING’ NATURE

Paris, where I resided in the 1970s, is halfway between the Ardennes Hercynian massif, where *L. helle* and its ecological companion, *P. eunomia*, are present, and the Morvan, where none of them had been found. I went back to my former fad: to try an ‘experiment’ in biogeography (Descimon 1976) by introducing individuals of both species from the former region to the latter one, where favourable habitats seemed to exist. Today, such a manipulation should not be allowed without presenting a serious research project, but anybody was allowed what he wanted to do by that time. The precise features of this experiment are presented elsewhere in this book, and I will only present its anecdotic side here.

With these introductions, I was granted a thrill of a new kind. It began when I carefully released the butterflies, in the most sheltered corner of the chosen habitat, and then looked at them beginning to activate and to lay eggs on *Polygonum bistorta*. However, it was in the following years that my heart was going pit-a-pat when I reached the places where the founders had been previously released. It was with *P. eunomia* in 1973 that I felt the emotion to observe the success of the introduction I practiced in 1970. This emotion came later with *L. helle* (during 1976-77), but it was not lesser.

Actually, since there was virtually nothing new to observe on the field of the distribution of *L. helle*, I replaced the question ‘is *L. helle* naturally present here?’ by the question ‘is *L. helle* able to be present here?’ However, with the only tools provided by field observations, the endless quest of ‘something new’ was limited to observing the extension of the two introduced species’ territory. In 1978, I became professor at the Provence University, Marseilles. The Morvan was much farther from this town than from Paris, and it was difficult to carry on field work within the narrow interval of time left free by my teaching duties. I therefore only loosely could follow this extension.

During the 1980s, things changed again. I looked for research subjects for my students. Now, the introductions of *P. eunomia* and *L. helle* and their genetic consequences were obvious ones. Actually, in these researches, *P. eunomia* soon appeared to be an easier and richer material. Allozyme electrophoresis (tempted in vain by Thierry Lelièvre) looked unexpectedly difficult in *L. helle*, while Bernard Barascud and Gabriel Nève readily mastered it with *P. eunomia*. In the field, the larger butterflies were easier to spot from a distance. And the extension of the Fritillary was much wider all over the Morvan than for *L. helle*. The Nymphalid therefore took precedence over its ‘smaller sister’. It was the subject of officially funded research programs and allowed the production of several theses and articles (e.g. Nève et al. 1996, Barascud et al. 1999), while, at that time, *L. helle* was given no more than a casual glance. However, things have completely changed today and the Violet Copper has taken its revenge and is the subject of a book!

BACK TO FIRST LOVE

At the opportunity of working with Philippe Bachelard about *Parnassius apollo*, I dug out the unpublished data I had accumulated all over the Massif Central, and, in 1999, we published a paper summarizing our observations in this region (Bachelard and Descimon 1999). By doing so, I believed that I took my leave to the Violet Copper. At the opportunity of working on *P. eunomia*, I was acknowledged that *L. helle* had invaded the upper and more southern region of the Morvan, the Folin massif, even before *P. eunomia*, which followed shortly later on. However, with the achievement of the researches about the Nymphalid, I had no more incentive to go to the Morvan; after my retirement, things became still more accentuate and the Violet Copper sunk into oblivion.

I was therefore stunned to receive an invitation from Jan Christian Habel to participate to a book about my little butterfly friend. It looked obvious that I had to check precisely the extension of the colony I founded 40 years ago and, in the end of May 2011, I rediscovered the mood I felt so long ago. But there were deep changes: instead of the flutter of coloured wings, which surrounded me in the olden days, there was here and there a scanty butterfly – often a *P. eunomia*. Most clearings were obliterated by scrub, dense conifer plantings had been performed almost everywhere; meadows were heavily grazed by a new race of cattle, which ensures better income to the rare breeders, which have not gone away to the town. The Morvan region has become a 'Parc Naturel Régional', a status which is purported favouring both economical development and nature conservation. Obviously, the second goal has been missed.

Moreover, spring had been so hot and dry that butterfly flight period was advanced by at least three weeks. However, I roamed desperately through this territory, so familiar to me forty years ago. Sometimes, I felt like those movie actors opening the door of what had been their house and being faced to a yawning chasm! The most distressing was to look for a clearing, which had been formerly spotted with precision in the forest and to wander without meeting any very least trace of it. After one week of fruitless search, in a still neglected meadow full of *P. bistorta* and Marsh Marigolds at l'Huis Prunelle, I met, at the end, with the well-known small glitter with an 'impossible colour', actually an old-timer *L. helle* male. I was therefore allowed to state that the Violet Copper was still there. In 2012, I again visited the Morvan, but in early May – foolish choice! This time, spring had been cold and chilly and the flight period was delayed by three weeks. I was faced with a butterfly desert, not even an Orange Tip or a green-veined White was to be seen. I went back to Marseilles having completely failed. I fear that, following the climatic hurry-scurry of recent years, *L. helle* has vanished from the place I introduced it 40 years ago, but the searching conditions which prevailed during 2011, 2012 and 2013 even preclude to draw a definitive conclusion.

Will I come back to the Morvan in the future? The observations I could make at this opportunity are likely to be negative. However, it remains an interesting question: Why was the elder sister of *L. helle*, *P. eunomia*, thriving in all the remnant marshy meadows of the Morvan? Anyway, this fact shows that both species actually are 'false twins', as already stressed by Marc Meyer (1982).

WHAT IS RESEARCH?

In the beginning of this sketch, I raised the question of what determines the pathway of scientific research. The present case is a minute instance of such a pathway, so the much that it has always been marginal in my activity; its scope could therefore be so much limited as being insignificant. On the other hand, this could also mean that it is free of the social constraints, which determine scientific research, especially at present time.

Clearly, it is a reductionist thesis to assume that research consists only of rational processes, independent of the real life of those who practise it. The *primum movens* of my interest for *L. helle* was not a research program, but a 'collector behaviour' (not to forget that collection has scientific and aesthetic components). No more was rational the poetic mood, which surrounds *L. helle* and its environment, which contributes to the attraction felt by the naturalist. This sentiment is shared with, for instance, mathematicians speaking of an 'elegant theorem' or physicists invoking a 'beautiful theory'. Further, events of my personal life were paramount in 'canalizing' my interest for *L. helle*. However, once these non-scientific determinants have oriented the choice of this research material, scientific reasonings based on a combination of empirical and theoretical considerations, such as the elaboration of the previously mentioned 'suitability index', were no more dependent of life casualties.

Before all, I guess that research is a game. To play, researchers mobilize all their resources, including physical force and endurance. In this deal, reasoning holds the major role, but imagination is necessary, as in any game. However, I think that something else is working in us at more depth, a kind of instinctive behaviour – one could name it 'the heat of discovery'. It is probably this behaviour, which inspired the human species to conquer the whole Earth, while the other apes huddled in their ecological niche. We, scientists, have the privilege to continue the conquest, even should it be through the pursuit of a minute purple and orange butterfly.

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The Violet Copper *Lycaena helle* at its northern distribution range

Nils Ryrholm

Department of Electronics, Mathematics and Natural Sciences, Faculty of Engineering and Sustainable Development, University of Gävle, S-801 76 Gävle, Sweden, E-Mail: nrm@hig.se

Key-words: Distribution, ecology, habitat, decline, northern distribution margin

ABSTRACT

The Violet Copper *Lycaena helle* reaches its northernmost distribution range in Fennoscandia, where the butterfly can be observed from sea level to the mountain ranges in Sweden and Norway. The limiting factors are (i) the occurrence and habitat decline of its single larval food plant *P. vivipara* (instead of *P. bistorta* as in Central Europe), and (ii) confinement only to sites with short and sparse vegetation producing warm microclimatic conditions, which allows a relatively fast larval development in the comparatively cold and wet Scandinavian climate. The butterfly was quite common over most of northern and central Scandinavia as long as agricultural activities were relatively extensive. However, the population has declined strongly since the Second World War. Since then, previously suitable habitats have either been abandoned and overgrown, or transformed into intensively used and fertilized agricultural land. Taken together this has led to a severe loss of *L. helle* habitats in farmed areas, and subsequent strong fragmentation of the remaining populations. The extremely cold and wet summer 1987 finally eradicated a vast number of already fragmented populations, which already were weakened due to low habitat quality and subsequent low population densities. However, not all the northernmost populations and those in the wettest regions went extinct, as could have been expected if the unsuitable weather as such had been the major cause for this mass extinction. The decline of *L. helle* still continues, and the today very rare butterfly can mostly only be found in small and isolated remnant populations. It appears as both the butterfly and its host plant today survive better on limestone influenced and (nectar-) flower-rich wetlands with sparse vegetation than on farmland. Presumably this was the primary habitat type in Scandinavia before ancient human agriculture made more and drier habitats available.

COLONIZING FENNOSCANDIA: THE PAST NORTHERN RANGE EXPANSION

The Violet Copper *Lycaena helle* reaches its northern distribution range in Fennoscandia. In this area, the butterfly occurs as subspecies *L. helle lapponica* (Backhaus 1876). Its wings



Figure 1: Female of *Lycaena helle* in Härjedalen, Sweden.

are slightly less vibrant in colouring compared with individuals from Central Europe (c f Figures 1 and 2 p 16-17; Figure 1 p 45 and page 146-151). The butterfly can be found over a large range of altitudes, from sea level to the tree line. Its single food plant is *Polygonum vivipara* (and not *P. bistorta* usually used over major parts of Europe). Molecular genetic analyses revealed that populations from Fennoscandia are closely related with individuals from North-eastern Europe. The Fennoscandian branch of a neighbour joining phenogram is nested within the eastern European samples. Furthermore, the Fennoscandian samples show a clinal loss of similarity to the individuals from Eastern Europe along the colonisation trajectory, from the south-eastern to the north-western parts. The successive loss of genetic information along this colonisation trajectory is a further signal that Fennoscandia was colonised from Eastern Europe, via Finland to the northern parts of Sweden and Norway (see Method article III and Article IV in this book).

PHENOLOGY AND ECOLOGY

The butterfly starts to fly at the leafing of birch species (*Betula* sp), which is, depending on year and latitude, usually from mid-May to the beginning of June. In years with a late season, the very last individuals can be observed in mid-July. After mating, the female lays the eggs at the underside of the leaves of the larval food plant *P. vivipara*. Approximately ten days later, the larvae hatch and start to feed on the plant during the



Figure 2: Male of *Lycaena helle* in Härjedalen, Sweden.

next weeks. The duration of the larval stage strongly depends on weather conditions and the microclimate at the ground; a strong solar radiation heating reaching the low plants and the ground is of high relevance to guarantee a high body temperature of the larvae. Warm microclimatic conditions are of importance for the successful development of *L. helle*, as otherwise the species will not be able to complete its larval development in such a harsh and cool environment. This means that, if the vegetation becomes denser and starts to hinder the sun from reaching and heating the ground, the host plant and the habitat will become unsuitable for larval development. The larvae pupate on the ground and the pupae hibernate until next spring.

Throughout its northern range, *L. helle* uses *P. vivipara* (L.) as its single host plant. This flower is a low plant that grows in low vegetation, but is rapidly outcompeted by bigger and / or taller plants if these can establish themselves. Within the range of *L. helle*, the plant is persisting on most habitat types with low vegetation. The species initial habitats most likely were sites with high water levels or moving ground water. These conditions prevented the growing of taller and wooded plants. It also appears that the plant is doing better in some areas with limestone rich ground and is more resistant here. With the starting of human agricultural activities, this plant started to spread over a number of habitats where human activities kept the vegetation low, such as pastures, meadows and road sides. Many of these sites are considerably drier than the original habitat types, but since the climate in northern and central Scandinavia is fairly wet there is only a minute risk of drying out for any plant species. Presumably the traditional hay

meadow regime was very suitable for *P. vivipara*. As the species name indicates, this species reproduces itself mainly vegetative with sprouts dropped from the lower part of the stem. When the hay was mowed in late July or early August and then harvested, these sprouts were easily spread.

In the traditional agricultural practice, which was common over major parts of Fennoscandia until the 1950ies, *L. helle* used to be a widespread butterfly species in the central and northern parts, from approximately 60 N in Sweden and somewhat further south in the western part of Sweden and in the Norwegian mountains, and then all the way northwards up to where agriculture was not possible. The species has occurred east of the Norwegian mountain range and the corresponding inland areas of central and northern Sweden on most sites where the host plant was available. The butterfly also occurred all the way (east) to the more intensely cultivated lowlands along the Gulf of Botnia, but here the species have always been more local and rare.

After the Second World War, the “ancient” extensive farming system (cf. Figure 3) changed rapidly in Sweden. Many remote and extensively used sites have been either abandoned or transformed from low-yield sites into intensively used units by artificial fertilization and ensilage production. Where the small scale mosaic was destroyed and the ground was fertilized, the plant community drastically changed in favour of larger, fast growing and more competitive species. This has lead to a gradual decrease in suitable habitats for the plant (and the butterfly) and this negative process still continues



Figure 3: Some traditional farms are still active in the Bruksvallarna area in Härjedalen. *Lycaena helle* still occurs in suitable habitats with moderate disturbance, like in the foreground of the picture. The picture is taken when the butterfly is on the wing and before the host plant is flowering, so these tiny plants are not visible.

until today. As a result of these changes, not only the host plant declined, but also the amount of suitable habitats with low vegetation vanished. As a consequence, already in the 1960s, the butterfly species became more and more scattered in the southern range, where it only occurred on agricultural areas, and as the land use changed and became more intensified fewer and fewer suitable habits were left. This negative trend led to the fragmentation of the remaining habitat patches and the populations living therein. Thus many populations became exceedingly isolated, and the major proportion of the butterflies' populations went extinct during this and the coming decades.

The "summer" 1987 was a climatic disaster and the coldest for more than a hundred years in Sweden. The temperature was several degrees below the average, and the sky was clouded most of the summer, allowing for extremely few sunshine hours. A large number of the already small and isolated populations of *L. helle* still present in habitats with reduced habitat suitability went extinct in that year, even though it took several years before the full extent of this disaster for *L. helle* was revealed. One would perhaps have expected that the populations in the coldest and wettest and the northernmost part of Scandinavia would have suffered most during this extremely cold period, but it was clearly the populations on the most deteriorated habitats that were least able to cope with these extreme weather conditions. Whether it was the direct impact of the weather on the survival of the larvae or its impact on farmer activities like leaving the hay uncut and to rotten on the ground, which was most detrimental for *L. helle*, remains an open question. Nevertheless, as a result the species disappeared from almost all of its sites south of 62°N (slightly less at higher altitudes) and also from most of the sites in the lowland areas close to the Gulf of Botnia. After this disaster, there is a slower but continuous process of retreating inland, upland and northwards for *L. helle*, leaving more and more of the (former) habitats on cultivated land (see Figure 4).

RECENT OCCURRENCE AND FUTURE TRENDS

By now (2013), the butterfly is not known south of 62.5°N on lower lands and 61.5°N in the mountains in Sweden. The major distribution area left is in the counties of Härjedalen, Jämtland and western Medelpad (see Figure 4). There are also a few observations along the coast of the Gulf of Botnia, between the cities Umeå and Luleå demonstrating that there are still some populations surviving within this region. Hopefully some populations still survive somewhere in the vast areas of the interior of Norrland that may be discovered before they are gone.

Today, the butterfly becomes more and more depending on the original wetland habitat types again (Figure 5). In Jämtland there are a large number of wetland areas influenced by limestone ground where the species still exist in relatively large populations. In this region, *L. helle* also occurs in between these sites along gravel road sides, under power lines and on military training fields, i.e. areas kept open by humans, but not used for any kind of "production". In Norway, the situation and distribution trends are simi-

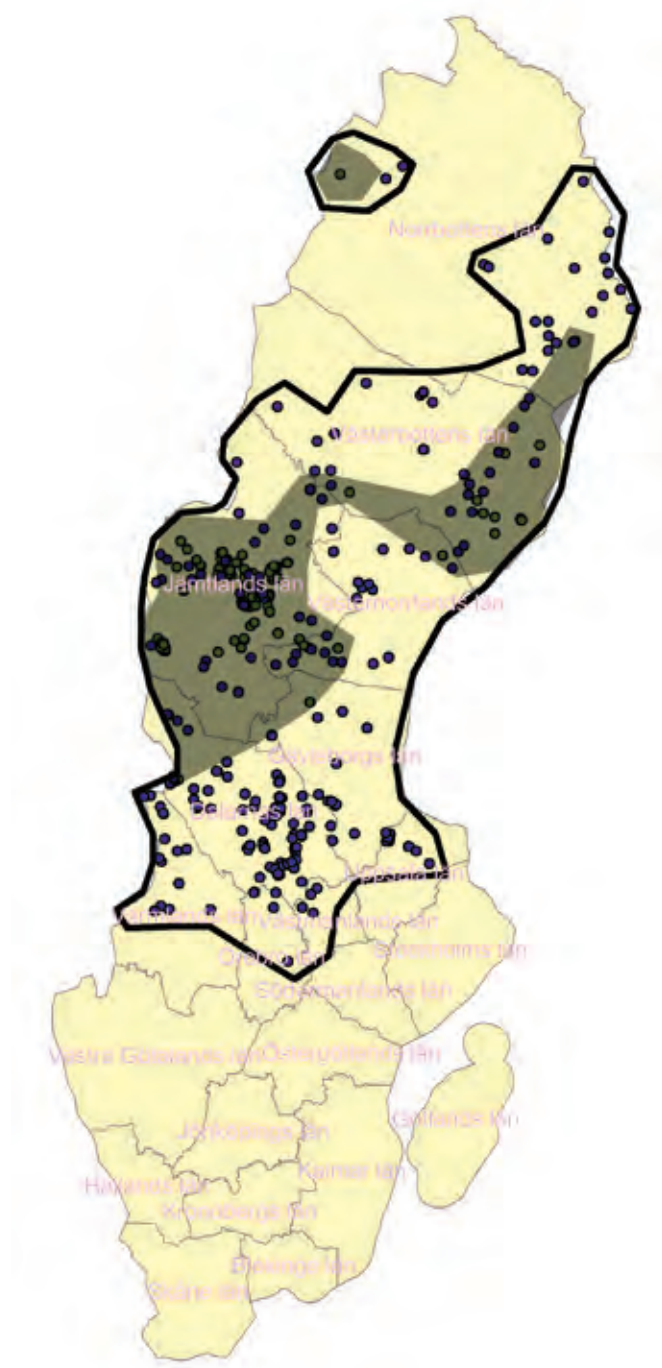


Figure 4: Distribution of *Lycaena helle* in Sweden. The black line delimits the maximal known distribution; the dots are all actual finds and the grey area is the presumed occurrence 2013. Compilation made by Mats Lindeborg.



Figure 5: Wetland habitats with low vegetation where *Lycaena helle* is still thriving. Marshland on limestone ground appears to be the natural habitat for this butterfly species in the Nordic countries. Despite the much wetter character of these habitats, the host plant is doing well also in this kind of habitat. When the artificial habitats in the boreal farmland are abandoned and consequently become unsuitable, the limestone rich marshes turn into the last resorts for the butterfly. However, even these habitats are gradually getting overgrown in many places.

lar as those found in Sweden. Due to the low number of entomologists in Norway, the knowledge of the occurrence of *L. helle* is less well known than in Sweden and Finland, thus it is difficult to give a more detailed description of the process. However, since the Norwegian agricultural policy is different by supporting small scale farming and human life in rural areas to a higher extent than in Sweden, the decline may have been a bit slower. Today, the butterfly is known from 6 to 8 localities in Norway in the counties of Oppland, Hedmark and Nord-Trøndelag, thus the centre of gravity for the presently known Norwegian distribution is slightly further south than in Sweden, presumably as a result of the higher altitudes prevailing in the region. Both in Norway and Sweden, the butterfly appears to persist better in areas with limestone ground or at least with strong influence of limestone. Hopefully, there are still also a number of undiscovered localities hiding on the still partly vivid Norwegian countryside.

The present status in Sweden can be seen on the ArtPortalen Homepage: http://www.artportalen.se/bugs/uttag_gmaps.asp?art=101248&art_leaf=False
And in Norway on the Artsobservasjoner Homepage: http://artsobservasjoner.no/smakryp/uttag_gmaps.asp?art=46590&art_leaf=True

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Habitat requirements, threats and trends in the distribution of the Violet Copper *Lycaena helle* at its northern distribution margin in Finland

Marko Mutanen* & Panu Välimäki

University of Oulu, Department of Biology, FI-90014 University of Oulu, Finland
*Corresponding author: Marko Mutanen, University of Oulu, Department of Biology,
P.O.Box 3000, FI-90014 University of Oulu, Finland, E-mail: marko.mutanen@oulu.fi

Key-words: Climate change, endangered species, habitat fragmentation, metapopulation

ABSTRACT

In this chapter, we examine trends in abundance and distribution of the Violet Copper *Lycaena helle* in Finland during the past century. The Violet Copper was considered as nearly threatened in 1985, but its status has exaggerated since then from vulnerable in 2000 to endangered in 2010 based on the IUCN criteria. The main criterion is the restricted area of occupancy that is likely to show continuous decline based on each of the five subcriteria defining population vulnerability. The species was protected by law in 2006 in Finland (Nature Conservation Act 160/1997), and it is included among the species that need strict protection in Europe (EU directive 92/43/EEC, Appendix IV). We describe local habitat requirements of the Violet Copper and propose possible explanations about the reasons and mechanisms that have led to its apparent decline. Some of our suggestions are well defined hypotheses and some rather wild guesses, but due to a lack of explicit studies, fairly speculative altogether. We conclude that loss and fragmentation of semi-natural grasslands underlie the decline of the Violet Copper in Finland, although other factors have likely acted in concert with habitat deterioration.

CHANGES IN THE DISTRIBUTION AND ABUNDANCE OF THE VIOLET COPPER IN FINLAND

The history of the Violet Copper *Lycaena helle* in Finland follows the same sad trend as observed elsewhere in Europe (Van Swaay and Warren 1999). Finland has a long tradition of butterfly monitoring by professional and amateur lepidopterologists. The species was recorded in Finland for the first time in 1788 in the biogeographical province of *Lapponia enontekiensis* in the north-western part of the country (Thunberg 1791) (see Figure 1). Later on, it has been reported from each province, except *Alandia* in the south-western archipelago (Kullberg 2004) (Figure 2a). The last observations from the



Figure 1: Finnish biogeographical provinces (Al= Alandia, Ab= Regio Aboensis, N= Nylandia, Ka= Karelia australis, St= Satakunta, Ta= Tavastia australis, Sa= Savonia australis, Kl= Karelia ladogensis, Oa= Ostrobothnia australis, Tb= Tavastia borealis, Sb= Savonia borealis, Kb= Karelia borealis, Om= Ostrobothnia media, Ok= Ostrobothnia kajanensis, Oba= Ostrobothnia borealis pars australis, Ks= Regio kuusamoensis, Obb= Ostrobothnia borealis pars borealis, Lkoc= Lapponia kemensis pars occidentalis, Lkor= Lapponia kemensis pars orientalis, Le= Lapponia enontekiensis, Li= Lapponia inarensis).

three southernmost provinces (*Nylandia*, *Regio Aboensis* and *Karelia australis*) close to the Baltic Sea go back to the 1930's, 1940's and 1950's, respectively (Pakkanen and Wettenhovi 2007). The decline of the Violet Copper in Finland only awoke attention as late as 1990 (Marttila et al. 1990), but even then it was assumed to persist in many areas where it likely had already become extinct. Eight years later, Wettenhovi and Kaila (1998) indicated, based on Atlas data on the Finnish Lepidoptera, that the Violet Copper had shown a rapid decline since the 1940's and that this decline continued until the 1990's. After the year 1998, the species has been reported from six of the 19 provinces where it had been observed before (Figure 2b).

Although already vanished from southernmost Finland and many other areas, the Violet Copper was still regularly reported from four more or less separate areas during the period 1978–1987, including a relatively large inland area of the southern part of the country (Huldén et al. 2000). During 1988–1997, no more records from this wide area were reported, indicating a dramatic decline and sudden collapse of this population network. Since this period, a small isolated population was rediscovered in southern *Tavastia australis*. The last known record of the species on that particular locality was made in 2002, although the patch has been regularly visited in later years as well (Blom-

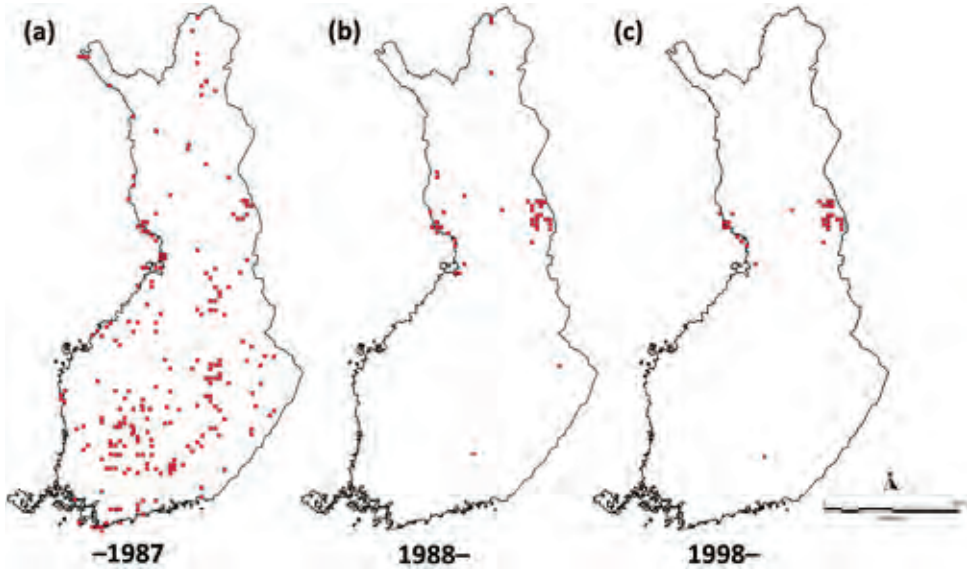


Figure 2: Distribution and decline of *Lycaena helle* in Finland. 2a. All records before 1987; 2b. Records 1988–1997; 2c. Records since 1998.

ster 2007). Currently, the population has probably gone extinct. During the same time interval (1988–1997), a few records were still made in eastern Finland *Karelia borealis* (last observation 1991) and the eastern part of northernmost Lapland *Lapponia inarensis* (last observation 1996) (Pakkanen and Wettenhovi 2007). Yet, the review of Pakkanen and Wettenhovi (2007) is not totally comprehensive as there are some unpublished records from *Lapponia inarensis* after that (E.M. Laasonen, personal communication). No doubt, the species has declined also in northern Finland and become extinct in many areas there. Accordingly, the range of the Violet Copper has been shrinking from the both margins, and many populations between the last persistent populations disappeared in the late 1970's or early 1980's. During the last decades, the species has been regularly reported from two separated areas in the provinces of *Ostrobothnia borealis pars borealis* and *Regio kuusamoensis*, which we later refer to as Tornio and Kuusamo, respectively (Figure 2c). Both these populations have been subjected to several surveys during the late 1990's and the 2000's. Surprisingly, the latter surveys have indicated that the Violet Copper has larger ranges and inhabits more numerous patches in both areas than supposed by the earlier studies.

Heino et al. (1998) investigated the network of the Violet Copper subpopulations in Kuusamo in 1997. They discovered only six occupied habitat patches in their study area, and did not manage to find the species from several patches where it was reported some years earlier. They estimated the population size in this network of habitats to be only 200–250 individuals at that time. While the results of the survey were alarming, they likely gave far too pessimistic perception of the reality. We and several other lepidopterists have observed the species in many localities over the Kuusamo region since then, including habitat patches separated by 20–30 km from the study area surveyed by Heino et al.

(1998) in the late 1990's as well as several occupied patches within their study area that were either not surveyed at all or considered as vacant at that time. Admittedly, some of these patches may have been colonized in the time in between. Moreover, even though not based on quantitative data, the population sizes in many patches have very likely significantly exceeded the estimations provided by Heino et al. (1998), who reported only relatively few individuals from each patch during each visit. We have observed dozens of individuals in some patches per visit, even within very limited areas. Therefore, the populations might have undergone an exceptionally poor phase at the time of Heino et al.'s investigation, but also the reported suboptimal weather conditions during their survey may have contributed to the unrealistically low population size estimates.

The Tornio area has been intensively investigated in 1999 (Soininmäki 1999) and by the authors of this chapter in the 2000's. The area of occupancy has been shown to be wider than presumed in this area. Soininmäki (1999) reported nine extant populations mainly around the municipalities of Tornio and Kemi, but the range extends both eastwards and southwards into the province of *Ostrobotnia borealis pars australis* about 50 km from this area. The southernmost, though possibly isolated and scarce, population is recently discovered from the municipality of Kiiminki in *Ostrobotnia borealis pars australis*, about 80 km south of Kemi. This population, which is the southernmost known persisting population in Finland, is regularly followed by the authors since 2004. The species has been observed by a single individual in three years only, and the population is possibly at the verge of extinction. Alternatively, the source population is yet to be discovered as there are several habitat patches seemingly suitable for the Violet Copper not systematically surveyed so far.

While the geographical range of the Violet Copper once extended from the southern coast of Finland to northernmost Lapland (Valle 1935), it is now, in practice, only sustained in two areas in southern Lapland. The known populations are, however, doing better than assumed by earlier investigations (Heino et al. 1998; Wettenhovi and Kaila 1998), and may possibly be even expanding. It is likely that undiscovered populations still exist in northern Lapland as the area is large and relatively poorly surveyed. However, despite rich occurrence of the food plant and seemingly suitable habitat as well as recent throughout investigations, the species has not been found in several areas in northern Finland, in particular *Ostrobotnia kajanensis*, around the municipality of Pello in *Ostrobotnia borealis pars borealis* and the village of Kilpisjärvi in *Lapponia enontekiensis*. The last known observations of the Violet Copper from *Ostrobotnia kajanensis* and Kilpisjärvi were made in 1971 and 1948, respectively (Krogerus 1972; Pakkanen and Wettenhovi 2007). A single individual from Pello was observed in 1995, but it is still possible that the species persists in that area. Populations, if any, are likely to be very local as we have not been able to find a single individual during the past five years when we have visited the area (and even that particular spot) regularly during the seasonal flight period of the species. Even if the Violet Copper seems to be doing well within its current area of occupancy, the species is rare and vulnerable to stochastic deleterious events because the total number of extant populations is fairly low in Finland.

HABITAT REQUIREMENTS AND LIFE HISTORY OF THE VIOLET COPPER IN FINLAND

The Finnish vernacular name of the Violet Copper (luhtakultasiipi) suggests that its preferred habitat are wet, swampy meadows. Based on the current knowledge, the vernacular name does not fit very well, and it has been speculated whether such wet meadows, perhaps natural or pastured riverside meadows, might have been its primary original habitat in Finland. In any case, this is not the situation any longer, and evidence that this has been the case in the past remains scarce (but see Valle 1935). The food plant of the Violet Copper, *Polygonum viviparum* (Heino et al. 1998), does not usually occur in very wet places, but rather on relatively dry semi-natural grasslands (Hämet-Ahti et al. 1984; Anderberg and Anderberg 2008). We doubt that the Violet Copper has ever inhabited moist or mesic meadows in Finland, at least not at that extent that would justify its vernacular name. The food plant prefers fresh to relatively dry meadows, but it clearly avoids dry stands with too permeable soil. However, *P. viviparum* grows on a certain type of treeless swamp on calcareous soil rich in nutrients (i.e. fen). Possibly the Finnish vernacular name refers to such habitat types.

Polygonum viviparum has declined over Finland (Lampinen and Lahti 2011, Figure 3) obviously because the area of meadows characterized by relatively low and sparse herbaceous vegetation has dramatically decreased in Finland due to changes in agricultural practices. The most prominent change in the present context is the decline of grazing of semi-natural habitats. Grazing maintains transition dynamics in plant assemblages and enhances the survival of herbaceous plants, especially those of the early successional stages (Olofsson et al. 2001). The area of semi-natural grasslands has been estimated to be reduced to less than 1% of their total area in the late 1800's (Pykälä 2001). Despite this dramatic decline, the food plant is still relatively common, though its abundance spatially varies a lot. It gets more common towards the North, and it is fairly abundant within the two core areas of the Violet Copper. *P. viviparum* is, however, notably common also in many areas where the Violet Copper does not occur any longer.

Polygonum viviparum is a weak competitor over space. Thus, the species declines due to overgrowing by taller grasses or shrubs. No doubt, the plant has benefited from grazing in earlier times. Road banks, which are regularly mowed, have partly compensated the loss of sustainable habitats for *P. viviparum*, and consequently most habitat patches of the Violet Copper are currently found on road sides. Besides that, it is obvious that *P. viviparum* prefers calcareous soils in northern Finland. Nowadays, the best known areas for both *P. viviparum* and the Violet Copper are all on areas with limestone, a mineral that is found in relatively few areas in Finland. In some places, like Kiiminki, the occurrence of *P. viviparum* very strictly follows soils enriched by limestone so that its occurrence stops abruptly when this effect ceases. Road banks not influenced by limestone (or other nutrients) typically do not form suitable meadow-like vegetation in northern Finland, although the plant occurs commonly on semi-natural grasslands and other more nutrient-rich places also in such areas.

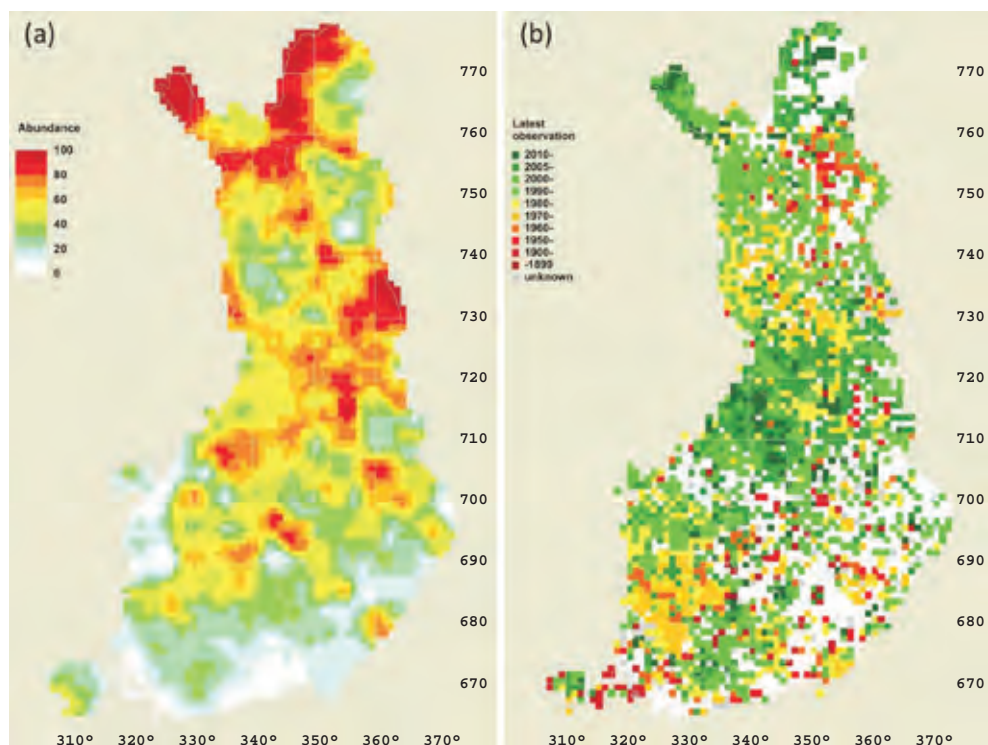


Figure 3: Distribution and abundance of *Polygonum viviparum* in Finland (after Lampinen & Lahti 2011). 3a. All-time abundance is based on the frequency of occupied 1 km × 1 km grid quadrats in each 10 km × 10 km quadrats (max 100); 3b. Time frame of the last record from each 10 km × 10 km quadrat.

The literature mentions several food plants for the Violet Copper (e.g. Marttila et al. 1990), but these are likely erroneous or refer to documented observations in other countries than Finland. In Finland, the species is strictly monophagous on *P. viviparum*. In their breeding experiment, Heino et al. (1998) observed a single larva feeding on *Polygonum aviculare*, but no one accepted *Rumex*. Accordingly, the occurrence of the Violet Copper in Finland is exclusively associated with that of *P. viviparum*. Females lay relatively large whitish eggs on the underside of the leaves, and the occurrence of the Violet Copper can be confirmed easily by inspecting leaves for eggs in June. Eggs are regularly laid on leaves that are not shaded by other vegetation, typically close to the edges of vegetation-free areas. Larvae complete their development in July or early August, and the species overwinters exclusively in the pupal stage (Seppänen 1969).

Adults emerge earlier than usually reported in the literature, in some years already in May even in northern Finland, and regularly at the beginning of June. The flight season usually ends by Midsummer, but may continue until early July in late years. Adults frequently visit flowers and, almost without exception, the habitats are rich of *Geranium sylvaticum*, which is obviously the preferred nectar source of the species in Finland. The

emergence of the adult butterflies seems to be synchronized with the blooming of *G. sylvaticum*. Curiously, in Kiiminki, the species is lacking in a road bank with extremely rich occurrence of *P. viviparum*, while is occurring along another road bank with lower densities of the larval food plant, but rich in *G. sylvaticum*. Consequently, nectar sources are vital for the survival of adults and partly determine the habitat use of the species.

This kind of response is likely among species whose fitness is affected by resources obtained at the adult stage (i.e. income breeders), but unlikely applicable to species that rely on larval-derived resources for reproduction (i.e. capital breeders). In the Glanville Fritillary (*Melitaea cinxia*), both immigration and emigration is affected by the abundance of nectar sources in a particular habitat patch (Kuussaari et al. 1996), while in the Common Blue (*Polyommatus icarus*), females prefer to oviposit on hosts that provide nectar for themselves (Janz et al. 2005). These examples demonstrate nicely that the distribution of adult resources may affect adult behaviour at different levels (habitat selection / oviposition site selection) in butterflies, and may even explain why adults sometimes make decisions seemingly suboptimal for the larvae.

POSSIBLE CAUSES FOR THE DECLINE OF THE VIOLET COPPER IN FINLAND: FOUR HYPOTHESES

It is undisputable that the distribution of the Violet Copper has strongly decreased during the last hundred years and that the remaining populations are highly fragmented in Finland. The decline started probably already before the 1940's and continued until the 1990's (Wettenhovi and Kaila 1998). Since then, the decline has stopped, at least in the light of observations. Meanwhile, the Violet Copper has occurred abundantly within its known currently fragmented range during the last years. Even though it is likely that the area of semi-natural grasslands such as pastures and other comparable meadows will show further reductions in the future, it has to be underlined that most patches occupied at the moment are located at road sides, which are not reducing. Possibly, these derived secondary habitats will provide sanctuaries for the Violet Copper that may support the long-term persistence of the species in Finland. In the following sections, we discuss potential causes that underlie the decrease of the Violet Copper. The suggested causes are not mutually exclusive, and we deem that the decline is a sum of several contemporary factors.

Factor 1: Habitat decrease and fragmentation

The traditional explanation for the decline of the Violet Copper is loss and fragmentation of its primary habitat (Heino et al. 1998; Wettenhovi and Kaila 1998; Rassi et al. 2010), which result in a collapse of metapopulation level processes and in local extinctions at some point. This inevitable outcome makes sense both intuitively and theoretically

as decreasing population sizes due to habitat loss increase the risk of local extinctions, while simultaneously increasing distances between habitats decrease colonization rates of vacant patches and eliminate immigration from source to sink populations (i.e. the rescue effect) (Gilpin and Hanski 1991). Indeed, there is strong evidence that both, the food plant and area of suitable meadows, have significantly declined and remaining patches have become more isolated in space in Finland during the last hundred years (Pykälä 2001; Lampinen and Lahti 2011).

This hypothesis is further supported by the fact that the two current core areas of the Violet Copper are especially rich of the species' habitat and the food plant. In fact, 60% of the Finnish butterfly species inhabiting primarily semi-natural grasslands have declined during the past five decades (Kuussaari et al. 2007). Owing to these, habitat reduction and deterioration undoubtedly is one of the key factors that have resulted in the decline of the Violet Copper. We, however, doubt that this process alone explains the observed decline because the Violet Copper has become extinct from many areas still rich of its food plant and habitat. In Kilpisjärvi, for example, its food plant is extremely abundant along riverside meadows prevented from overgrowing over time especially by grazing of semi-domesticated reindeer, which has been shown to benefit herbaceous plants and the associated lepidopteran fauna (Välimäki 2005). Similarly, both habitat and food plant are still fairly abundant in *Ostrobottnia kajanensis*, about 200 kilometres southwest of the core area of Kuusamo. Despite recent efforts, the once relatively common Violet Copper has not been observed for four decades.

The decline of the Violet Copper is comparable to the yet more dramatic decline of another lepidopteran species, *Capricornia boisduvaliana*. This species once common almost throughout Finland is now known from a single patch close to the municipality of Rovaniemi in *Ostrobottnia borealis pars borealis*, although it was fairly common in *Ostrobottnia borealis pars australis* still in the late 1970's (Välimäki and Itämies 2002). The situation is the same in Sweden, where only one population is known (Ryrholm and Ohlsson 1999). Indeed, to our knowledge, these two populations are the only ones persisting in Europe West of the Ural Mountains, and the species is considered as critically endangered both in Finland and Sweden (Gärdenfors 2010; Rassi et al. 2010).

There are significant correlates between the decrease of the Violet Copper and *C. boisduvaliana*. Both are living on structurally similar semi-natural grasslands with relatively sparse and low herbaceous vegetation and have likely benefited from grazing or mowing. Both have had their strongest populations roughly at the same geographic areas. Both species are known to have co-occurred in the same meadows, although *C. boisduvaliana* is exclusively feeding on *Trifolium pratense* (Välimäki and Itämies 2002).

The occurrence of *C. boisduvaliana* in its previous range has been a subject of several recent thorough investigations (Välimäki et al. 2009), but these have not yielded new discoveries. A significant difference is that while the Violet Copper has managed to inhabit secondary habitats, notably road banks, that seem to compensate for habitat loss, this is not the case in *C. boisduvaliana*. Seemingly suitable habitats for *C. boisduvaliana* are typically found along road sides in northern Fennoscandia, but both per-

sistent populations still inhabit isolated meadows produced by traditional agriculture. Notably, the trials to re-establish *C. boisduvaliana* on suitable-looking meadows close to its former occurrences have yielded promising results as introduced populations have, at least, temporarily established. This may be an indication that habitat loss alone might not explain the extent and rate of decline of *C. boisduvaliana*, although the statement of fragmentation effect remains vital. If there is another cause, we argue that it might be the same in both the Violet Copper and *C. boisduvaliana*, since the decline of both species happened more or less simultaneously.

Factor 2: Climate change

It is well documented that the Finnish lepidopteran fauna has undergone a rapid change during the last two decades so that dozens, if not hundreds, southerly species have expanded rapidly towards the North (Pöyry et al. 2009). Even though the reverse pattern in the south border of species' ranges is more difficult to document, there are indications that the southern edge-of-range of many species has shifted towards the North (Viidalepp and Mikkola 2007; Pöyry et al. 2009). The Violet Copper has historically always had a northerly distribution in Finland (Valle 1935). It thus appears possible that climatic conditions in the southern areas of the Violet Copper's range have been suboptimal during the last decades when the climate has been rapidly warming (Finnish Meteorological Institute 2008). We therefore assume that climatic factors play at least some role in the drawback of the Violet Copper's range. This hypothesis, however, can hardly explain the collapse of northernmost populations.

Seasonality that appears as regular cycle of favourable summer and adverse winter affects insect life histories especially in the northern latitudes (Kivelä 2010). This is because only a part of the year is suitable for insect reproduction and growth. Insect life histories have to match with prevailing environmental conditions as diapause is usually restricted to a species-specific developmental stage that has to be reached before the conditions turn adverse (Danks 1987). In the Violet Copper, diapause is restricted to the pupal stage, and the species is predominantly univoltine in Finland (Marttila et al. 1990). The species has, however, genetic propensity to bypass diapause and some individuals may thus develop directly into the adult stage within the same season (i.e. to express bivoltine phenology). This is highlighted by the fact that, during an exceptionally warm summer, emergence of a (second) summer brood was observed as far North as Tornio in southern Lapland (Finnish Entomological Database 2011). We argue that, in Finland, the emergence of the summer brood is detrimental even in warm summers. Summers are simply too short, and hence the potential for exponential population growth due to bivoltinism does not become realized, but the opposite where offspring of summer generation are invariably doomed to death due to the forthcoming autumn frosts.

We even argue that the genetic tendency for direct development may partly explain why the species has practically never occurred in southernmost Finland close to the coast

line of the Baltic Sea, where relatively long summers may induce direct development and result in a mismatch between life history and environmental conditions. Similarly, increasing season length along with warming climate may underlie the decline of the southernmost populations that have become extinct by now. We stress that actually a single year with exceptionally favourable conditions for direct development might be enough to result in local extinction as long as a large enough number of individuals develop directly without a possibility to contribute to the diapause generation [fitness accumulates as geometric series, no nils allowed (see Seger and Brockman 1987)].

A possible example of mismatch between life history and environment is provided by the congeneric Small Copper *Lycaena phlaeas* in Finland. The species is strictly univoltine in the North (ssp. *polaris*), but obligatorily multivoltine in the South (ssp. *phlaeas*) up to the southern edge of the biogeographical province of *Ostrobothnia borealis pars borealis* (Marttila et al. 1990). Interestingly, there is an apparent 300 kilometres gap between the populations where the species does not occur at all (Finnish Entomological Database 2011). A likely explanation is that the univoltine population cannot cope with increasing season length (offspring survival is not guaranteed under extended periods of pupal diapause under warm conditions), whereas the southern population cannot expand northwards as bivoltinism is not an option north of the current edge-of-range.

The above reasoning demonstrates that the season length may have potential to shape species' geographical ranges depending on species' life history. Whether this holds true in the case of the Violet Copper or not has to be assessed in future research. Either way, climate warming and subsequent changes in average temperatures and season lengths should not be ignored as a potential threat to Lepidoptera. Thus, further studies concentrating on species' ability to respond changing climate are warranted.

Factor 3: Casual disaster

While the decline of the Violet Copper appears to have started from the southernmost populations and then proceeded gradually to the North, this picture is rough and not fully supported by existing data. For example, the species seems to have disappeared from *Ostrobothnia kajanensis* in Central Finland before it vanished from inland areas of southern Finland. Although difficult to document, we cannot exclude the possibility of a single or several especially disastrous years in the past. This disaster might be due to especially poor weather conditions during any stage of the life cycle. A single year with all or most offspring of spring (diapause) generation emerging within the same season followed by an early onset of winter or even a single severe autumn frost might potentially cause fatal damages to population sizes as explained in the previous section. A similar result would follow, if extremely poor weather conditions prevail during the adult stage rendering reproduction impossible. This option requires, however, relatively restrictive assumptions because such a weather "catastrophe" should cover a large enough area simultaneously or otherwise vacant patches would be recolonized by immigrants from

the surrounding populations. Moreover, such a phenomenon should result in simultaneous extinction of many nearby populations, which is not the case in the Violet Copper characterized by a gradual decline over decades.

Alternatively, populations of any species may be swept down by biological agents, in particular parasitoids, bacterial diseases or endoparasitic bacteria (*Wolbachia*) infestation. Yet, we are not aware of such factors in the Violet Copper. Intracellular lifestyle of *Wolbachia* imposes significant limitations on its ability to spread as it can only be transmitted vertically from parent to offspring through cells in the female germ line. Thus, it can spread from one population to another only if carried by immigrants. More importantly, *Wolbachia*'s success is necessarily positively correlated with the reproductive success of its host. If the pathogen becomes more harmful and decreases host fitness, it is less likely to be passed on to the following generation. Owing to this, vertical transmission tends to evolve benign pathogen–host interactions despite of the possible initial virulence. Indeed, *Wolbachia* infected insect females tend to produce more offspring than uninfected ones, and hence *Wolbachia* may spread rapidly within a host population. Owing to limited dispersal ability and strong selection for decreasing virulence, *Wolbachia* hardly underlie rapid decline of spatially structured host population within a large geographical area.

However, the above explanation for population decline that relies on detrimental biological agents may be more likely applicable to the decline of *C. boisduvaliana* than that of the Violet Copper. The two persistent *C. boisduvaliana* populations are the northernmost populations ever known and remarkably isolated from the historical core area or any other known sites of occurrence, which would be expected because the spread of any biological agent depends on habitat connectivity. To the contrary, the Violet Copper has persisted especially in the core areas, local extinctions being notable especially among edge-of-range populations.

Factor 4: Pesticides and fertilizers

DDT was largely used as an insecticide in Finland during the period 1953–1977. One might assume that its extensive use might have had negative effects in many insect species at least at a local scale. The time period of extensive use of DDT fits well with the decline of the Violet Copper. The use of DDT was prohibited by law in Finland in 1976. While DDT decomposes slowly, its current effects are supposed to be minor, and curiously, the decline of the Violet Copper seems to have ceased during the past two decades. Still, we consider unlikely that the DDT might have had anything but a partial role in the decline of the Violet Copper.

Various fertilizers, especially those containing nitrogen, are nowadays omnipresent in agriculture. The use of nitrogen favours plants such as Nettles (*Urtica dioica*), which are not characteristic to traditional semi-natural grasslands. Based on our extensive mapping of such meadows while searching for populations of *C. boisduvaliana* in central and northern Finland in 1998–2009, we noticed that suitable meadows are practically

lacking in areas with active agriculture. While overgrowing is predominantly resulted from decrease of pasturing and mowing, it is further aggravated by the use of fertilizers, not to mention reforestation of former grasslands. Both have parallel very negative effects on species requiring semi-natural meadows such as the Violet Copper. We are convinced that fertilizers are among the negative factors behind the decline of species, the underlying mechanism being habitat loss and fragmentation.

CONCLUSIONS

It is indisputable that the Violet Copper has declined drastically in Finland since the 1940's. Once distributed nearly throughout the entire country, the species is now largely restricted to two core areas in southern Lapland. However, there is evidence that this decline has not continued after the 1990's. Indeed, based on more or less regular monitoring, these two populations form seemingly viable metapopulations with many relatively individual-rich sub-populations. In Finland, the Violet Copper typically inhabits habitats sustained by traditional agricultural practices such as semi-natural meadows and pastures. The total area of such habitats has reduced considerably in Finland, and thus it is evident that habitat deterioration and fragmentation is a major reason of the decline. Nevertheless, this is hardly the sole explanation as the Violet Copper has vanished from several areas with seemingly inhabitable habitats. We therefore argue that several detrimental effects have acted in concert, including climate change, frequent use of fertilizers in agriculture among other possible causes.

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The Violet Copper *Lycaena belle* in the Pyrenees: Distribution and ecology at the species' southern distribution margin

Graham Hart^{1*} & Nick Bowles²

¹Le Sauzet, F-09310 Chateau Verdun France

²94 Miswell Lane, UK-Tring Herts HP23 4EX, United Kingdom

*Corresponding author: Graham Hart, Le Sauzet, F-09310 Chateau Verdun France, E-mail: pappyren@aol.com

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Abstract

The southern distribution margin of the Violet Copper butterfly *Lycaena belle* covers parts of the Pyrenees. Its distribution was studied in 1995 and 1996, and again in the year 2012 by reviewing the literature and extensive field studies. We compiled occurrence data of the species and measured the habitat suitability and population density by egg counts along transects and by recording squared plots. We found a population cluster in the Pyrenees Mountains at an area of about 500km². The second field campaign in 2012 indicated a severe decline in population sizes. The ideal oviposition situation is of a mid range moisture content, vegetation of a heterogeneous structure with about 10 to 20cm height and the presence of 'apparent' leaves of the larval food plant *Polygonum bistorta*. The eggs are always laid in a warm microclimate situation usually in hollows in the vegetation. The presence of tufts or tussocks of grass with lots of dead leaves around the tussock, or dead leaves of *Juncus* and *Carex*, seems to be of high importance. The main reasons for this severe population decline are believed to be due to over grazing on the one hand and abandonment on the other. Suitable management regimes are rotational grazing or cutting and removal of large tussocks to produce an earlier successional stage. A habitat improvement and restoration project based on this knowledge was started in autumn 2012 at the two main study sites where *L. belle* was very common in the year 1996. These conservation actions are conducted in collaboration with farmers and landowners.

OCCURRENCE OF *LYCAENA BELLE* IN THE PYRENEES

Lycaena belle is highly local and restricted in the Pyrenees and is only known from the eastern part of these mountains, covering an area of about 500km² in the French De-

partments of the Pyrénées-Orientales (Capcir, Cerdagne and Porté Puymorens), Ariège (Donezan) and at the edge of Andorra (La Solana d'Andorra). *Proclossiana eunomia*, the ecological sibling taxon of *L. belle*, is also found in this region, but shows a much wider distribution around this central area where it flies with *L. belle*. In the following we highlight (i) the main occurrences of *L. belle*, (ii) the habitat demands of *L. belle* for oviposition and to live as imagos, and (iii) discuss population trends in areas where we performed our ecological studies.

Department of Ariège

In the valley of the Donezan, about 1000m to 1500m asl, more than 40 habitat patches were discovered in 1996. At that time, almost all suitable habitat patches were occupied. In 2012, the majority of previously identified habitat patches rested unoccupied (see below). Most suitable habitats at 1000m were unoccupied until today, while new colonies could be found at higher altitudes, at an elevation between 1800m and 2000m, which were previously not searched in 1996.

Department of Pyrénées-Orientales

In Capcir (1500m to 1900m asl), 21 habitat sites have been discovered including also very large habitat patches. In Puyvalador, Formiguères (Barascud pers. comm.), the species can be found in the valley bottom and all the way up to the ski stations. Further habitats and populations can be found near the following villages: Matemale (most of the way around the lake), Caudier de Confluent, Railleu along the Ruisseau de Railleu, Les Angles, La Blagonne, Mont Louis at Col de Quillana (Reale 1962), and at Lac de Bouillouses. In Cerdagne (1600 to 1900m asl), Eyne (Barascud pers. comm.), Pyrenees 2000 Ski Station, Fort Romeu, Angoustrine. At Porté Puymorens in the valley bottom and up at the ski station, also south along the valley towards the Cerdagne at Porta, Val de Camcardos, Cortvassili and close to Latour de Carol.

Department of Pyrénées-Orientales (Andorra)

Further populations (discovered by GH in 2011) exist at the bottom of La Solana d'Andorra; Pla de la Vaca Morta (Andorra), next to the river on the north side of the valley above L'hospitalet pred l'Andorre and also on the south side of the valley (Pyrénées-Orientales) below the road N20 running up to Pas de la Casa between 1600 and 1800m asl. Most of the suitable habitat is found on the south side of the valley. Also last year (2013) other populations were found above the road N20 between the road and Porté Puymorens Ski station (Demerges pers. comm.).

TRANSECT COUNTS: PRESENCE, ABSENCE AND HABITAT SUITABILITY ASSESSMENT

GH performed 12 transect counts of *L. helle* at five different locations in the year 1996. NB repeated six of these transects at the two most important localities in the year 2012 (three transects at each site). Additional transect counts were further conducted at apparently still good sites in the Pyrenees Orientales by NB, in the north of the Capcir at Puyvalador, about 5 km distant from the sites studied in the Donezan valley and at Porté Puymorens.

Eggs of *L. helle* were counted in 10m² plots along a 20m long transect (counting every other meter) using a quadrat of 1m². The quadrat was subdivided into 25 compartments of 20 × 20cm by using string. All vascular plant species were recorded per quadrat including their abundance by estimating their cover using the DAFOR scale (dominant, abundant, frequent, occasional, rare). We further recorded the number of leaves of the larval food plant *Polygonum bistorta*. For every leaf on which eggs of *L. helle* could be found, we measured the length and width of the leaf as well as the height above ground where the egg was oviposited. The position of each egg was indicated in the respective vegetation map. Measuring the height of vegetation was performed applying the Boorman disc (BUTT 1986), recording maximum and minimum Boorman disc heights for each quadrat; from these data we obtained a maximum and minimum vegetation height index (not the mean vegetation height). We further measured pH value and moisture content at the first and last quadrat of each transect in the year 1996. For some egg oviposition sites, we measured the temperature at the leave surface, as well as the temperature at the surrounding vegetation.

In the year 1996, we counted up to 46 eggs in a 10m² plot, and 33 in very dominant *Molinia caerulea* tussock dominated habitat where *P. bistorta* was just hanging on but at high apparency between tussocks (Table 1). The results for the still good sites in the Pyrénées-Orientales in 2012 are less than one third of the best results in 1996. In consequence we have to ask: Was 1996 just an exceptional year? Was 2012 rather a bad year? Or is this a rather worrying alarm call to take action at all of these sites? These questions will be addressed in detail below.

HABITAT QUALITY AND OVIPOSITION

There are two distinct situations in which eggs are laid, and they occur in a variety of vegetation communities. The first was open and sunny, often at grass tussock or tuft edges where : there was dead organic material available (grass *Juncus* and *Carex* species). The second, again sunny, was in hollows in taller vegetation, especially amongst *Filipendula ulmaria*. In fact, the female seems to be using the same cue in both of these situations, and that is high apparency of the *P. bistorta* leaves, also noted in other species such as *Hamearis lucina* (Porter 1992). ‘Apparent’ is a term coined to describe leaves which are in some way distinct within the habitat and attractive to the female as oviposition sites (Porter 1992).

Table 1: Egg oviposition and the ecological conditions along different transects, including the following parameters to describe the habitat structure and quality: number of leaves, number of eggs oviposited, pH-value at the respective site, moisture of the soil, mean size of leaves, mean of the maximal vegetation height (calculated over all plots), and mean of the minimum vegetation height (calculated over all plots) in 1996.

Transect No / Site Name	<i>N</i> leaves analysed	<i>N</i> eggs	pH soil	Moisture (%)	Leaf size (cm ²)	Veg. height Mean max. (cm)	Veg. height Mean min. (cm)
1-Noubals	74	33	6.1	62	36	41	9
2-Noubals	2123	26	6.3	57	45	34	25
3-Noubals	944	42	6.1	54	73	41	16
4-Noubals	814	2	6.3	82	40	34	22
5-Noubals	1995	29	6.1	55	87	46	29
6-North of Noubals	396	3	6.2	57	142	86	55
7-Mouilleres	851	46	6.1	55	36	27	11
8-Mouilleres	1019	37	6.2	23	37	22	12
9-La Linas	968	11	6.0	77	50	47	29
10-Querigut	1290	0	5.7	75	~	23	18
11-La Mas	673	12	6.2	86	60	26	19
12-Querigut 2	1978	0	6.4	19	~	46	35

In the case of *L. helle*, it seems equally valid to describe the leaves which are attractive as unobstructed. Observations showed that females of *L. helle* were simply choosing broad, flat (45° to horizontal) leaves, which in some way stood out from the 'background noise' of other vegetation. Broad leaves with visual separation of some kind between them and the background against which they were viewed were strongly favoured over both apparent narrow leaves and obstructed broad leaves. It could be that the frequent choice of leaves above litter was because they were more apparent against the pale background.

Various aspects can affect apparency, such as vegetation height and vegetation structure. Where vegetation height as measured by Boorman disc is up to about 35cm, the *P. bistorta* leaves can grow to the top of the vegetation, improving their apparency. Even at 35cm, the most apparent leaves will be the ones in hollows in the vegetation where they grow more horizontally (i.e. more apparent). In taller vegetation, the *P. bistorta* leaves are only apparent in hollows. Note that some of the best areas had a maximum Boorman disc vegetation height of 40 to 45cm, i.e. tussocks of *M. caerulea*, but with large hollows between these tussocks where the *P. bistorta* grew often in a very apparent fashion in sunny but sheltered situations.

The ideal vegetation structure suggested by our results has a maximum Boorman disc vegetation height of 20cm and a minimum of 10cm, i.e. a heterogeneous composition. Further important habitat characteristics are vegetation hollows providing warm microclimates in which the leaves of *P. bistorta* occur with high apparency and an abundance of dead organic material on the ground.

The density of *P. bistorta* leaves may affect apparency. Where *P. bistorta* grows in high density, the plants tend to grow nearly vertically and thus are of low apparency. However, the leaves at the edge of the clump tend to spread out more, growing more horizontally, in other words with higher apparency. Isolated plants and small clumps have a higher proportion of apparent leaves and are more favoured for egg laying. Eggs were nearly always laid on the southern side of the plant.

The difference in apparency in managed and unmanaged meadow situations is important. Cutting a meadow seems to reduce the size of the *P. bistorta* leaves growing the following year, so they are lower in the vegetation as well as being more densely packed, rather vertically and having a lot of tall grass stems growing up between the leaves, i.e. this leads to low apparency of the leaves. Furthermore, there is no layer of dead organic material (leaves of grasses, *Juncus* or *Carex* species) in managed meadows. In the unmanaged meadows, there is a build-up of dead organic material, the leaves of *P. bistorta* were larger, often growing more horizontally, and free from grass stems, i.e. of high apparency.

Apart from the apparency of the larval food plant, further parameters influence oviposition; microclimate, soil moisture, and the presence of dead organic material: *L. helle* oviposited in habitats with a wide range of soil moisture content, from about 20% to 85%. However, fewer eggs were laid in the wetter part of this range. The optimum range for soil moisture content seems to be 20% to 65%, as indicated by the presence of *L. helle* in most of those habitats providing these conditions. The presence of a layer of dead organic material seems to be an additional important parameter, particularly in situations with soil moisture content of over 75%. This may be to provide an ideal microclimate for larval development as seen in other species such as *Argynnis adippe* (Warren 1993).

Larval development was certainly found to be fast in this study, and leaves on which eggs were laid were several degrees warmer than ambient temperature. The warmest places of the area were directly around where eggs were laid. However, the actual egg laying position was often 15cm or more above the dead organic layer. At this distance, the temperature effect would be rather limited. Another explanation would be the presence of a suitable overwintering site for the chrysalis. In the transects with the most eggs there was a lot of dead organic material in form of tufts and tussocks of grass, which seem a likely site for the chrysalis, however, direct evidence is still missing. One larva reared in captivity on a pot containing *P. bistorta* and a small tussock of *M. caerulea* pupated at the base of a live grass blade growing up out of the base of the tussock.

In conclusion, the optimum oviposition habitat for *L. helle* has to provide a high abundance of dead organic litter of grass, tufts or tussocks (or *Carex* or *Juncus* species), a heterogeneous vegetation height between 5cm and 20cm (maximum 40cm) with *P. bistorta* growing in apparent situations (hollows, tussock edges, situations providing a warm microclimate) and a mid-range soil moisture content.

HABITAT QUALITY AND IMAGOS

The presence of trees, particularly glades and scallops in woodland edges with the presence of tall plants such as *Veratrum album*, *F. ulmaria* and grass tussocks, are necessary as perching sites for the territorial males. Preferred are sites with woodland or scrub on at least two but preferably three sides, where males often form leks. The degree of territoriality depended on the size of the site, at small sites with a low adult density and scarce male territorial resources (perches) the males are highly territorial. GH observed up to 11 males occupying one woodland glade next to a large open area of habitat suitable for oviposition. At another site, a very extensive open area with a copse of trees, the copse was open in the centre two male *L. helle* were holding territories. Furthermore, trees are used as roosting sites at night.

In addition to these rather structural habitat parameters, the presence of nectar sources is important particularly for females, particularly for females. In the sites studied in the Ariège during the first half of the flight period, *Myosotis scorpioides* (which was often locally abundant) was the most important nectar source, while it was *P. bistorta* in the second half (when it started flowering). A variety of other nectar sources were also used according to their abundance. Males and females were observed to nectar side by side without any courtship interactions in the morning (9h to 10.30h).

Oviposition habitat is often spatially isolated from mate location habitat; the females wander over open habitat with the larval food plant *P. bistorta* growing in situations as described above. The furthest distance from trees that an egg was recorded was 400m. In a large forest area in the Ariège, an egg was found in a 25 × 5m glade that was separated from the nearest other glade by 500m of high forest. In the same area, two other eggs were found on *P. bistorta* plants growing on the side of a sunny forest track several hundred metres from the nearest known colony. In one particular area in the Pyrénées-Orientales (Southern Capcir), extensive open pine forests with many glades and *P. bistorta* growing in ideal apparent situations often associated with grass tussocks, *L. helle* exists as a low density population all over this area with mate location and oviposition both occurring in the glades.

POPULATION DECLINE IN *LYCAENA HELLE*

Sixteen years ago, *L. helle* could be found in many places in the Donezan valley in the Department of Ariège. Males were often found holding territories some way from oviposition habitat. This suggests that the population was strong, and the competition between males for the best territories was forcing some of them to use territories of suboptimal quality (Dennis 2010). In this valley, GH identified five important medium to large colonies, which were considered important for the maintenance of the entire population network.

On visits to the valley over the last three years, males were totally absent from territories of suboptimal quality. This might be interpreted as an effect due to a population decline. Population assessments at all five major sites in the year 2010 showed that the butterfly was absent from one site, and occurred in much lower densities at the other four sites. In 2011, when the species became extinct at a second site, we started to repeat the transect counts that were performed by GH in 1996. In 2012, NB continued this work, including also sites from the Pyrénées-Orientales where the butterfly is still found in high densities. In the Ariège, along one transect where GH recorded 33 eggs in 1996, only three eggs could be found in 2012. At another site where GH counted 46 eggs in 1996, no eggs could be found anymore in 2012 (and only one egg could be found after one hour by assessing all plants which seemed to be of high suitability for *L. helle* but outside our plots) (Table 2). Three adult *L. helle* were seen flying at this site in 2012. For the small habitat areas dotted all over the valley, most sites had changed radically, often now being totally dominated by *F. ulmaria* or rapidly changing into woodlands. Consequently, most of these stepping stones, which acted as important linking patches among the large and suitable habitats, were lost. The transects undertaken at the still favourable sites in the Pyrénées-Orientales at Puyvalador and Porté Puymorens in 2012 revealed a maximum transect count of 17 eggs, much lower than the maximum of 46 some 16 years ago; yet, these sites still hold apparently good populations of adult *L. helle*.

The question arises why the decline is so remarkable in the Ariège, but not (or less) in the Pyrénées-Orientales. Several factors seem to interact here. The weather in the Ariège is somewhat more “Atlantic” than that in the Pyrénées-Orientales (being more “Mediterranean” and lying on the other side of the water shed of the Pyrenees even though they are geographically adjacent). The woodland in the Ariège is mixed broad leaved and pine, predominantly *Fagus sylvatica* and *Betula* spec. with *Abies alba* and *Pinus* spec.; in the Pyrénées-Orientales, it is predominantly *Pinus* spec. with some *Betula*. In the Ariège with its rather wet climate and extensive forest cover, ecological succession seems to occur faster than in the Pyrénées-Orientales so that some areas have already become wet woodland, and in other areas *F. ulmaria* or *M. caerulea* have become completely dominant with no trace of *P. bistorta*. It seems that the sites in the Ariège are changing faster than in the Pyrénées-Orientales.

Comparing the two main study sites in the Donezan, Noubals and La Moullière du Pla and the Pyrénées-Orientales

Sixteen years ago, some parts of the site in Noubals (ungrazed in 1996) were already completely dominated by *M. caerulea* with no other plants growing between the dense and large tussocks, another area (also ungrazed in 1996) was completely dominated by *F. ulmaria*. However, another part about two hectares in area was still used by *L. helle* for oviposition. This area itself was a mixture. In parts it was dominated by tussocks of *M. caerulea* with very few but very apparent leaves of *P. bistorta* growing either between tussocks or out of

Table 2: Numbers of leaves searched, eggs located and ratio of eggs per leaf (data taken from Hart 1996, and NB).

Transect No / Site Name	N leaves analysed 1996	N leaves analysed 2012	N eggs total (per leaf) - 1996	N eggs total (per leaf) - 2012
Noubals A	74	129	33 (0.446)	4 (0.031)
Noubals B	2123	773	26 (0.014)	0 (0)
Noubals C	1995	292	29 (0.145)	0 (0)
Noubals D	814	246	2 (0.004)	2 (0.008)
Mouilleres C	1019	273	37 (0.036)	0 (0)
Mouilleres E	851	270	46 (0.054)	0 (0)
Puymorens meadow A	-	863	-	2 (0.02)
Puymorens meadow B	-	337	-	11 (0.032)
Puymorens Ski above A	-	105	-	7 (0.066)
Puymorens Ski above B	-	164	-	17 (0.100)
Puymorens Ski below A	-	498	-	1 (0.002)
Puymorens Ski below B	-	550	-	3 (0.005)
Puyvalador	-	358	-	13 (0.036)

the sides or even on tops of tussocks (transect egg count in 1996 = 33). In other areas, the *M. caerulea* was less dominant with a mix of nectar sources between tussocks and a lot of *P. bistorta* present. A lot of the *P. bistorta* leaves were growing very upright and densely packed, but the leaves were growing much more horizontally at the edges of these clumps so that they were quite apparent leaves for oviposition (transect egg count in 1996 = 42). In other parts, *M. caerulea* was absent; instead *Carex acutiformis* and rather stunted *F. ulmaria* were dominant; this habitat had plenty of hollows with apparent leaves of *P. bistorta* (transect egg count in 1996 = 26). A fourth and final type was abundant stands of *P. bistorta* with a mix of some few other abundant species, *M. scorpioides*, *Holcus lanatus*, *Agrostis stolonifera*, but no *M. caerulea*. This area looked like a carpet of *P. bistorta* with the other species coming up between the leaves. The vegetation height was fairly uniform with the grasses being taller than the *P. bistorta*, which was growing very densely and upright, i.e. of low apparency. However, the *P. bistorta* leaves were able to spread out more in places where the grasses were less dense, occupying more apparent conditions (transect egg count in 1996 = 29).

In 2012, a fence dividing off the ungrazed *M. caerulea* and *F. ulmaria* areas had been removed. The same parts of the site are still completely dominated by *M. caerulea* (when given a choice, the gazing animals ignore these areas), but the other areas have rather changed. The dominant *F. ulmaria* area of 1996 now has much less *F. ulmaria*, which is very stunted, and there are a lot of medium sized tussocks of *M. caerulea*. In an adjacent woodland glade that supported the largest lek of *L. helle* males, *F. ulmaria*, which had formed the most important perching sites in 1996, has completely gone leaving some *V. album* as the only suitable perches now with a maximum of three male *L. helle* being seen at one time in the last three years (the maximum was 11 males 16 years ago). This change in the *F. ulmaria* area has been caused by grazing at a quite high intensity and has gone



Figure 1: Male of *Lycaena helle* in the Pyrenees.

through stages where it became much more suitable for *L. helle* as the vegetation height reduced and became much more heterogeneous to the state today where over-grazing by cattle and horses (the latter even during the flight season) has caused a lot of poaching, grazing off of *P. bistorta* leaves during the flight season and even the destruction of tussocks leaving a flat short grazed turf in many places, which is totally useless for *L. helle*.

The main part of the site utilized by *L. helle* in 1996 is also being over-grazed, often leading to severe poaching between tussocks of *M. caerulea* so that there are just pools of open water between tussocks, and the dead organic litter between tussocks has virtually disappeared. The *P. bistorta* plants also appear stunted, the leaves are much smaller than the plants 16 years ago and the number of leaves in apparent situations is much less. *L. helle* still uses this area for oviposition, but the number of eggs found on the repeated transects here has fallen by over 90% (1996: 33 eggs, 2012: 3 eggs).

In the parts where *M. caerulea* was much less dominant 16 years ago, it has now become a lot more dominant. Marked poaching due to grazing pressure between the tussocks has caused much smaller *P. bistorta* plants with much lower numbers of suitable apparent leaves for oviposition. Also the numbers of nectar plants available have reduced significantly (transect not repeated in 2012). The area of *C. acutiformis* and *F. ulmaria* growing with *P. bistorta* has transformed to a much more uniform height with less hollows (1996: 26 eggs, 2012: zero eggs). Also here, the *P. bistorta* plants are now smaller with densely growing upright leaves, much fewer apparent leaves for oviposition, where we found just two eggs (searching for likely looking plants, not on the transect). These places were associated with the very few grass tussocks still remaining. The area



Figure 2: Female *L. helle* laying an egg on the underside of a leaf which is growing horizontally and is unobstructed i.e. it is highly apparent. Note also the presence of lots of dead organic material in the background.

of abundant *P. bistorta*, with fine grasses and *M. scorpioides* but no *M. caerulea*, was moderately poached and all the plants extremely stunted, the vegetation structure was totally changed and not suitable for *L. helle* any longer (1996: 29 eggs, 2012: zero eggs)

The site at La Moullière du Pla is over one kilometer long and quite narrow. Parts of it were very wet with invading trees, mostly *Betula* and *Salix* species, with lots of open glades in between. This area was good for male territories. However, as it was very wet underfoot with little *P. bistorta* but a lot of *Succisa pratensis*, it was not used for oviposition. The adjacent area of perhaps a couple of hectares of drier ground with plenty of *P. bistorta* was more suitable for oviposition (transect egg count in 1996 = 37). In the first part, this area was covered with grass tussocks then by a mix of meadow flora typical of the area (*A. stolonifera*, *Alchemilla vulgaris*, *Anthriscus sylvestris*, *Centaurea nigra*, *Crepis* spec., *Dactylis glomerata*, *Festuca rubra*, *Gallium* spec., *Geranium palustre*, *Holcus lanatus*, *M. scorpioides*, *Narcissus poeticus*, *Potentilla erecta*, *Rumex acetosa*, *Sanguisorba officinalis*, *Taraxicum officinale*, *Trollius europeus*, *Veronica chamaedrys*). The vegetation height was very heterogeneous with plenty of hollows with apparent leaves of *P. bistorta*. In another part of this site, the vegetation consisted of low tussocks of *M. caerulea* (tussocks only about 20cm high) with *P. bistorta* common throughout, but also *Carex binervis*, *Cirsium palustre*, *Festuca rubra*, *Gallium* spec., *Juncus* spec., *Plantago lanceolata*, *P. erecta*, *Potentilla reptans*, *S. pratensis* (very occasional) and *Viola palustris* (transect egg count in 1996 = 46).

Now in 2012, the invading woodland has progressed somewhat but is still suitable for male territories; however, the flower rich area next to it has been spring grazed by three or four horses over recent years. The sward is now grass dominated and hardly a flower is in sight, it is of very uniform height with very little tussock formation. *P. bistorta* is still common, but it is now being shaded by the dense grasses (1996: 37 eggs, 2012: zero eggs). The area that was lightly cattle grazed 16 years ago is abandoned today. The *M. caerulea* tussocks are now very big and dominant, few other plants remain, and there is a marked paucity of nectar sources. The *P. bistorta* plants still present have rather small leaves, often growing upright and often obstructed by grasses; rather few leaves were of high apparency. One adult female was found flying in this area, but we were unable to find any eggs (1996: 46 eggs, 2012: zero eggs).

In 2012, NB undertook several transects to compare egg numbers with the results in the Ariège and to the 1996 results. By that time, *L. helle* was found in every likely place surveyed all along the valley of Puyvalador in the Capcir, a vast high valley with a huge area of damp meadows. One of the best and easily accessible sites was at the side of the reservoir at Puyvalador. In 2012, the *P. bistorta* leaves here were larger than in the Ariège; sometimes they were associated with *M. caerulea* but often with other grasses. Visually, the site appears not to have changed since 1996 and this site has been the most consistent for finding adult butterflies in reasonable numbers over the last few years. NB estimated the area of suitable oviposition habitat at about 14 hectares, and eggs were relatively easy to find. However, only 13 eggs were found on a transect, but 24 eggs were found during a timed one hour egg search. Over two days, 15 adults were counted, the highest number for any site in the study in 2012, most of these were at just two lek areas.

At Porté Puymorens three areas were studied, firstly in the meadows in the valley bottom, a well known site. Most of the valley bottom was grazed in 1996, principally by horses, but a few adjacent meadows were only lightly and intermittently grazed (winter and early spring). Even today, small tussocks of grass prevail over most of this lightly grazed area and the *P. bistorta* plants are rather small. As the tussocks as well as the *P. bistorta* are rather small, a lot of *P. bistorta* leaves growing out of the sides or tops of these small tussocks were quite apparent. These meadows appear to have changed very little since 1996. One adjacent meadow has been abandoned and is in an ideal state for *L. helle* at the moment with large plants of *P. bistorta* in a moderate and heterogeneously high sward. However, there are a lot of saplings of mostly *Betula* species, so this meadow could suffer the same fate as a lot of other abandoned meadows at the southern side of this valley which are now Birch forests with *P. bistorta* hanging on in quite shady conditions totally unsuitable for *L. helle*. In about an hour's searching on likely looking plants, we found over 60 eggs. However, NB found just two and eleven eggs, respectively, on two transects carried out here; a considerably lower density than in the Ariège 16 years ago.

Above the road, at the entrance to the ski station the oviposition habitat was again dominated by tussocks of *M. caerulea*, but these tussocks are not too dense and there are a lot of medium to small *P. bistorta* plants growing out of or between the tussocks in good apparent positions. This habitat does not seem to have changed since 1996. However, we

also discovered areas like in parts of Nubals in the Ariège where *M. caerulea* was totally dominant, and no *P. bistorta* could be found. Consequently, we saw the whole range here, from little grass and very big *P. bistorta* plants through dominant *M. caerulea* with good sized *P. bistorta* plants to more dominant *M. caerulea* with smaller *P. bistorta* plants and finally to totally dominant *M. caerulea*. The transect results here were the best in 2012: two transects were carried out with seven and 17 eggs, respectively.

Below the same road, *M. caerulea* was a lot less common, and *P. bistorta* plants were big with their leaves often densely packed and vertical and plenty of heterogeneity in sward height with hollows with apparent leaves of *P. bistorta*. Eggs were relatively easy to find by searching likely looking plants, but again the transect numbers were disappointing: two transects were carried out resulting in one and three eggs, respectively.

DISCUSSION

Lycaena helle uses habitats that are in a mid-successional stage. Unmanaged suitable habitat in the Pyrenees lasts from about ten to 30 years, and probably longer in the areas of more Mediterranean climatic influence and at higher altitudes. Traditional management of the habitat has been extensive grazing, in spring and autumn at lower altitudes, but mostly summer grazing at higher altitudes. The animals used are cattle and horses. Grazing has drastically declined over the last 50 years. In 1996, there was just one herd of 65 suckler cattle left in the Donezan valley (within living memory in 1996, the number of cattle on farms in the Donezan was in excess of 600 (Blanc pers. comm.)). However, farmers from the foothills bring their animals up in summer to graze. They only use areas with easy access hereby causing over-grazing in some areas whilst others with difficult access or small patches of habitat have been abandoned. Many of these areas abandoned in the 1960's to 1980's were just at the right successional stage in the mid 1990's when the first study was carried out; the *L. helle* population was at a peak probably never seen before. Now, succession has progressed and many of these sites are no longer suitable as described above. The same phenomenon has been observed in other regions with *L. helle*; in the Ardennes after the Second World War (Waeyenbergh pers. comm.) more recently in the Eifel region of Germany (Fischer pers. comm.) and other areas of France (Descimon pers. comm.).

As succession continues, the taller plants in the vegetation tend to become dominant. So in some parts of the habitat (for example at Noubals), *M. caerulea* has become totally dominant with the build-up of a thick layer of its dead leaves in between tussocks, apparently stopping the germination of seeds hidden below in the superficial layers of the soil. In other areas, *F. ulmaria* has become highly dominant, the occasional plants of *P. bistorta* and *V. album* can be found just hanging on in these stands, and even dead tussocks of *M. caerulea* exist. Further succession sees the invasion of shrubs and trees changing the habitat in a more permanent fashion.

Effects of grazing

Grazing has profound effects upon vegetation height and structure and therefore on the suitability of the habitat for individual species of butterflies (BUTT 1986). Grazing at different stocking levels at different times of the year and with different species and even with different breeds of live stock will all produce different effects upon the vegetation (Oates 1993). Modern farming involves large herds and very few people to manage them, summer grazing areas are often heavily overgrazed, vegetation height is very low, *P. bistorta* is still present but the leaves are very small. There is a complete loss of dead organic material on the ground, which in the very wet areas is highly correlated with egg laying of *L. helle*. However, if there are no small tussocks or tufts of grasses or other plants, these areas are not used at all by *L. helle*. As an example, grazing pressure from cattle was high at the southern end of the Donegan, the areas with *P. bistorta* growing in were very wet habitats without organic layers. Consequently, *L. helle* was absent from this area. In many meadows at Porté Puymorens and southwards towards Latour de Carol with heavy summer grazing pressure from horses, the meadows were much drier than in the Donegan, the turf height was very low, a maximum of 5cm and often just 1 to 2cm. The tiny plants of *P. bistorta* were growing flat or virtually flat, and no or very little dead organic litter was on the ground. *L. helle* was not using this habitat.

The most dramatic example observed was a way-leave for an electric power cable. Across the way-leave was a barbed wire fence, which formed the lower limit of the summer grazing pastures. Above the fence, the vegetation was very short, *P. bistorta* plants were very small and no eggs of *L. helle* were found. On the other side of the fence, the vegetation was not grazed, it was of very heterogeneous height in the range of 10 to 20 cm tussocks of grass and also *Carex* leaves with an abundance of *P. bistorta* plants and many very apparent leaves. Eggs of *L. helle* were easy to find here and abundant.

The main study site in 1996 (Noubals) was spring and autumn grazed by the local herd of cows. This was for one month in autumn (October) and for up to 2 months in spring (mid-March to mid-May). The grazing area was not confined just to the *L. helle* site but went up the side of the mountain, so the grazing pressure could be said to have been light. In 1996, this grazed area of bog was much better for *L. helle* than nearby ungrazed areas, which were totally dominated by either *M. caerulea* or *F. ulmaria* (i.e. the succession was too far advanced). The grazing seemed to have the effect of breaking up the dead leaf layer between tussocks of *M. caerulea*, hereby encouraging the germination of seeds. However, there was no evidence that the cows were eating significant amounts of *M. caerulea* leaves, either dead or fresh green shoots. The other effect of the cows was on the *F. ulmaria*, which they will eat, thus breaking up the stands of *F. ulmaria* (trampling effect as well as eating) and stunting its growth in spring. The effect of this grazing produced a heterogeneous vegetation height and structure with apparent leaves of *P. bistorta* in ideal positions for oviposition by *L. helle*. Over the last few years, grazing pressure has increased and horses have been left there on occasional grazing in summer. This has led to the above described extensive poaching and general degradation of the



Figure 3: Noubals, the most important site in the Donezan with *Polygonum bistorta*.

site. Grazing prescriptions for such rather wet sites must be very carefully planned and executed to avoid dramatic declines in *L. helle* populations.

Cows do not eat *M. caerulea*, at least not to a significant extent. This is consistent with many other observations of cattle grazing other coarse grasses such as *Brachypodium pinnatum* (Oats pers comm.). The situation with horses is very different. At all three sites with horses, they grazed the green foliage of *M. caerulea* tussocks in 1996 virtually right down to the main body of the tussock; the remaining green grass blades were only 5 to 10cm long. Where the grazing pressure was not too high and *P. bistorta* was growing at the base of or out of the side of the tussocks, these plants had very apparent leaves and were frequently used for oviposition by *L. helle*. However, with higher grazing pressure, most of the apparent leaves of the *P. bistorta* had been grazed off and consequently there were no eggs of *L. helle*. In 2011 on one of these sites hardly grazed that year, ten eggs were found in less than an hour's searching. In 2012, the same site had been heavily spring grazed leaving hardly any apparent *P. bistorta* leaves; no eggs were found.

It therefore seems possible that cows and horses could be used in strategic ways when managing sites of *L. helle*. Where over dominant *M. caerulea* is a problem, then horse grazing is indicated to break-up the litter layer and reduce the dominance of the tussocks at the same time. If *F. ulmaria* is a problem, cattle grazing is indicated. Also careful grazing using both species could be used to try to maintain a site at the right successional stage.



Figure 4: Porté Puymorens (Andorra - Pyrenees Orientals) next to the ski station, tape measure for line transect in place.

We must emphasize that we are looking at two situations for grazing; restoration management and maintenance management and it is imperative to draw the distinction between these two. For restoration management, a period of intensive grazing would be needed throughout the greater part of the grazing season. This may need to be done for more than one year depending on results. Maintenance grazing must be at a lower stocking density and just for a part (or parts) of the grazing season. If animals are put on for a very short period in early spring, their trampling effect as well as a little grazing is beneficial for helping to maintain a heterogeneous vegetation height with hollows with apparent leaves of *P. bistorta* in. Autumn grazing is known to be beneficial for a number of species of butterflies, and is done at a time when *L. helle* is in the chrysalis state, probably well down in the vegetation and relatively safe. Further work is needed to study in detail the effects of different grazing pressures, timings etc. in the Pyrenees. In general, grazing requires the extensive use of fencing and a high degree of cooperation from the farmers to be able to put the animals on at the right time, in the right numbers, and very importantly, take them off at the right time also. Making specific grazing prescriptions therefore is very difficult in reality.

If there is one message to remember about *L. helle* and grazing it is that grazing is detrimental and over grazing, especially with horses and in the flight season leads quickly to local extinction.

As stated earlier, *L. helle* uses habitats at a mid-successional stage. This successional stage has often found to be difficult to manage (*H. lucina* and *Argynnis aglaja* for other



Figure 5: Two apparent *Polygonum bistorta* leaves with eggs.

examples). It seems that *L. helle* may be no exception at least in certain habitat types. At the relatively dry meadows in the valley bottom at Porté Puymorens, light horse grazing and occasional cattle grazing seem to be keeping the habitat stable, but there is a real problem with poaching and habitat succession in wetter situations. Furthermore, there is the ever present problem of liaising with the farmers in the area and persuading them to graze poor quality pasture as we want, when their main goal is to get there animals to grow as quickly as possible on better quality land. So grazing, especially restoration grazing, may necessitate the purchase of the necessary animals to be able to do as we would like, a very complicated and costly option.

However, let us have a closer look at the succession in *M. caerulea* associated habitat. As *M. caerulea* becomes more dominant, more and more plant species disappear (including importantly nectar plants) until we have just *M. caerulea* with *P. bistorta* hanging on, with nice apparent leaves of *P. bistorta* between tussocks and relatively high numbers of eggs of *L. helle* being laid. Then, presumably, the *P. bistorta* plants will die at some stage, and the mat of dead *M. caerulea* leaves on the ground prevents the germination of new plants, so we end up with just *M. caerulea*. If we cannot get hold of a load of horses, fence the site etc., what can we do? Just like coppicing for species such as *Boloria euphrosyne* in Southern England, we could manually (or mechanically, if the resources are available) take out the tussocks, thereby going back to a much earlier successional stage, light will get down to the soil, seeds will germinate and the whole cycle will start again. After some time maybe two to five years) we will again have suitable *P. bistorta*



Figure 6: Meadow at Porté Puymorens, in the centre the measuring tape for the transect.

plants mixed in with nectar sources and small tussocks of *M. caerulea*, the ideal habitat stage for oviposition by *L. helle*. Then, we can try to use light grazing to put a brake on the speed of succession and help us maintain our heterogeneous habitat. As time goes on and the *M. caerulea* again becomes too dominant, we start the cycle over again.

We have to accept the fact that we cannot maintain all the habitats all the time in optimal condition for *L. helle*, that we will need to manage on a cyclical basis and the butterfly will move between habitat patches as they become suitable. Consequently, the over-grazed sites in the area of Porté Puymonrens and at the southern end of the Donezan could be managed by making temporary enclosure by fencing off areas of fields say 5m wide for the length of the field or grazing pasture (probably acceptable for the farming community) and just leave it un-grazed for a few years. The *P. bistorta* already present as stunted plants in the sward will grow up as will everything else. In two to five years, we will again have our heterogeneous structure ideal for oviposition of *L. helle*. Then, as time goes on, we make other exclosures, ready to take over from the first ones as succession advances. We then take down the fence (electric fencing would be the most appropriate) from the first enclosure, the animals again graze the area, probably too hard and it goes back to a much earlier successional stage.

The observations on succession in *M. caerulea* dominated habitats raises an important question for the population found above the road at the Porté Puymorens Ski Station, now very good but no longer grazed (a fallen down barbed wire fence was found as evidence of past grazing). As time goes on, how long will it be before

P. bistorta dies out at the site and so the population becomes extinct? So, should we try to start some rotational grazing or taking up tussocks or disturbing the soil to encourage germination of new plants? If we start now, we can save this colony before it crashes.

Population density

Lycaena helle seemed to be existing in two different density situations. The majority of sites studied had large areas of open oviposition habitat, and males were often found in leks of up to eleven. These leks were closely associated with woodland edge, especially scallops and also glades within woodlands. Females were seen a lot less than the territorial males. Using a Mark Release and Recapture (MMR) technique, peak population at Noubals was estimated at about 400 individuals in 1996. The population estimate for the site over the whole flight period was estimated at 2000 to 2400 individuals. On a daily basis, that was about 200 adults per hectare (on the transect for the MRR, the daily counts varied from 33 to 46 butterflies, about 75% males and 25% females).

In some areas, the butterflies were at much lower density. Particular examples are at the southern end of the Capcir in an extensive open Pine forest and La Solana at the top of the Ariège valley. Adult density was much lower and the habitat structure was very different. These sites had no large open areas of highly suitable oviposition habitat as at the other sites, but the butterfly seems to be stable at both sites existing in diffuse low density populations. Apparently, *L. helle* can exist at low density if there are widespread and frequent small habitat patches (glades in open forest), but more commonly it takes advantage of the juxtaposition of large areas of damp grassland and woodland to form the larger denser colonies with which it is classically associated.

This pattern of localized high density colonies and in other areas diffuse low density distribution is also seen in other butterfly species such as *B. euphrosyne* and *H. lucina* in England. *B. euphrosyne* exists as localized dense colonies in the South-East of England due to the localized nature of its habitat (in coppiced woodland) and much more diffusely in the West of England, in Wales and in Scotland. *H. lucina*, like *B. euphrosyne*, used to be seen at low density over large areas of England in the early twentieth century, but with the abandonment of coppicing, the two butterflies declined dramatically, now being very localized, but at high density. So, these two species have had to adapt. One may say that natural selection has favoured individuals that are sedentary because the butterflies that have a tendency to migrate hardly ever find suitable habitat away from the site, so never produce offspring. Over time, the species has become more and more sedentary. This also seems to be the case with *L. helle*. Thus, Tulture et al. (2009) found females to be rather sedentary in the Ardennes where the species has become rather localized. On the other hand, Chuluunbaatar et al. (2009) found that the females are more mobile in Mongolia in a much larger diffuse habitat area, similar to our findings in the Pyrenees

where an egg was recorded in a little glade surrounded for at least 500m by tall forest. This suggests that where mobile butterflies are not selected against, the species as a whole will be more mobile hereby contributing to the health of the metapopulation. In areas with isolated large colonies, we should attempt to create stepping stone habitat areas between sites. This will increase the survival and so selection of more mobile individuals, which will increase gene flow, reduce the likelihood of genetic bottlenecks and favour a viable metapopulation structure.

Conservation action

Over the last three years, GH was been working with the Ariège Naturalists Association (ANA) on the decline of *L. belle* in the Ariège; Vincent Lacaze from the ANA has spent many hours putting a rescue plan together. The project has been awarded a grant of 170,000 € by the French Government for us to carry out. Forestry contractors will clear wet woodland from previously open sites where *P. bistorta* is still hanging on, including parts of La Moullière du Pla. The removal of tussocks of *M. caerulea* to create habitat of an earlier successional state and encourage the germination of *P. bistorta* seeds as discussed above, is being carried out at Noubals and at La Moullière du Pla. This work started in September 2012 with the help of 46 students. 5 × 5m squares of tussocks are being taken out. We are looking at four ways of managing the tussocks; i) Just brush cutting close to the tussocks and leaving the dead organic material. ii) Just brush cutting and taking out as much dead organic material as possible to aid germination of seeds. iii) Manually removing 50% of the tussocks. iv) Manually removing all of the tussocks. If time permits we will also try taking the top half off of tussocks. If time permits, we will also try taking the top half off of tussocks.

We also intend to control over-grazing at Noubals and the horse grazing at the meadow at La Moullière du Pla. Discussions are being held with local farmers (there are now several with at least small herds of animals in the valley) with the aim of having the right grazing regimes and also of targeting small sites near the large sites that were highly suitable 16 years ago, but are now dominated by *F. ulmaria* so that some cattle grazing should work well. Controlled grazing will also be used at one of the two important sites where *L. belle* has become extinct since 1996 (La Linas 1350m). The aim is to put four highland cattle in for a very limited time in spring to try to break up the uniform sward height (a lot of *F. ulmaria* is present) with a longer period of grazing in the autumn as is recommended by all other studies. The creation of grazing exclosures is planned at the southern end of the Donezan valley. At the same time, GH is in contact with two entomologists working in the Natural Park (Natura 2000 site of Porté Puymorens) Pyrénées-Orientales, discussing putting in grazing exclosures there and possibly reopening habitats that has now become wet woodland. We aim to continue the transects each year for several years to monitor the effects of our management and adapt our management prescriptions as necessary.

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Habitat suitability models in conservation planning – a short introduction

Petra Dieker^{1,2*}, Angela Taboada^{3,4} & Henrik von Wehrden^{2,5}

¹Institute of Landscape Ecology, University of Münster, Heisenbergstr. 2, D-48149 Münster, Germany

²Leuphana University Lüneburg, Centre for Methods, D-21335 Lüneburg, Germany

³University of León, Area of Ecology, Department of Biodiversity and Environmental Management, Campus de Vegazana s/n, E-24071 León, Spain

⁴Leuphana University Lüneburg, Institute of Ecology, D-21335 Lüneburg, Germany

⁵Research Institute of Wildlife Ecology, A-1160 Vienna, Austria

*Corresponding author: Petra Dieker, Helmholtz Centre for Environmental Research - UFZ, Department of Community Ecology, Theodor-Lieser-Straße 4, D-06120 Halle, Germany; Leuphana University Lüneburg, Centre for Methods, Scharnhorststraße 1, D-21335 Lüneburg, Germany, E-Mail: petra.dieker@uni-muenster.de

1. DEFINITION AND AIMS

In nature conservation, basic knowledge of species' habitat requirements is needed to develop effective management measures which will mitigate and counterbalance the effects of environmental change. Habitat suitability models (HSMs) have become fundamental tools to delineate the functional relationship between the occurrence (i.e. presence/absence or abundance) of a given species and the environmental conditions determining its distribution throughout a study area (Guisan and Zimmermann 2000). Great progress has been made in theoretical and statistical research and in computational techniques, and HSMs are now commonly used to predict suitable habitat for a wide range of species and are increasingly applied to a variety of challenges in ecology, conservation biology and biogeography (Schröder et al. 2009). These challenges encompass predicting the effects of climate and land-use changes on species' distribution patterns, designing reserve networks and restoring ecosystems based on key-stone species (Guisan and Zimmermann 2000; Guisan and Thuiller 2005). In this method box, we focus on the most important steps and critical issues for investigating species-environment relationships using HSMs – from the initial phase of the conceptual model formulation up to the application of model predictions.

2. CONCEPTUAL FRAMEWORK

The first step in building an accurate HSM is to match the overall goal and hypotheses of the study with the underlying ecological theory and the relevant modelling approaches. In the initial phase of the modelling procedure, the following conceptual aspects have to be considered:

- (i) *Fundamental versus realized niche*: Differentiating between the fundamental and realized niche is crucial to an understanding of the geographic distribution of species as well as the explanatory pattern of the models (Hirzel and Le Lay 2008). The fundamental niche of a species is determined by the species' physiological tolerance and the existence of favourable conditions (Hutchinson 1957, 1978; Sobéron and Pettersson 2005). The realized niche represents a reduced subset of the fundamental niche as a result of biotic interactions. In general, models based on empirical field data are likely to predict the realized niche of a species (Guisan and Zimmermann 2000).
- (ii) *Equilibrium versus non-equilibrium*: Static models assume equilibrium, or at least pseudo-equilibrium, between species' distribution patterns and the environmental conditions. However, the premise of non-equilibrium is often a closer match to reality because data collection is limited to a certain time and space, describing only a fraction of the species-environment relationship. In addition, many populations at shifting range limits violate the equilibrium assumption due to highly fluctuating environmental conditions (Guisan and Thuiller 2005; Braunisch et al. 2008). Therefore, dynamic, process-based modelling approaches are often superior to static ones (Zurell et al. 2009).

3. PRACTICALITIES

3.1. Scale

Selection of the proper spatial extent (e.g. continental, regional, landscape, habitat) and resolution (i.e. grain size) at which observations and environmental data are gathered is a critical step in HSMs (Guisan and Thuiller 2005). The spatial scale of analysis should always match the species' ecology in order to predict true responses to environment alteration; it should also take into account the respective species' life history (e.g. larval and adult life stages) and dispersal abilities. In addition, the spatial scale of HSMs applied to conservation planning should be relevant to management actions and enable an adequate selection of reserve sites (Seo et al. 2009; Bocedi et al. 2012). Also, the temporal scale (i.e. the temporal span that the study covers) is often crucial in determining the distribution of a species (Wiens 1986; Kneitel and Chase 2004), as in the case of species with seasonal or annual generations which have different environmental requirements.

3.2. Pre-selection of predictor variables

The predictive power of an HSM depends largely on whether the main environmental gradients determining the species' occurrence within the study area are captured by the predictor variables measured. In general, three types of predictor variables can be distinguished (Austin 1987; Guisan and Zimmermann 2000): (i) consumable *resources* (e.g. nectar and food plants of butterfly adults and larvae), (ii) *direct gradients* affecting the species' physiology (e.g. temperature, precipitation), and (iii) *indirect gradients* of no physiological significance for the species (e.g. topography: slope, aspect) that are easily measured in the field and consistently reflect the combination of different resources and direct gradients. The use of resource and direct predictors ensures model generality and applicability over large areas, whereas using indirect predictors reduces model transferability (e.g. Randin et al. 2006; Murphy and Lovett-Doust 2007).

Incorporating predictor variables which represent biotic interactions and species' dispersal abilities into the HSM is expected to increase the model's predictive power, since these parameters shape the species' occurrence and geographic distribution (Wisz et al. 2013).

3.3. Sampling design and data sources

Ideally, an appropriate sampling design that allows for sufficient hypothesis testing should cover the entire range of environmental gradients at which the species occurs. Depending on the aim of the study, the available time and financial budget, the following field sampling strategies are recommended (Guisan and Zimmermann 2000; Hirzel and Guisan 2002):

- (i) *Regular sampling* (e.g. grid sampling for distribution atlases): This approach is both time- and cost-effective since no initial environmental information is needed.
- (ii) *Random-stratified sampling*: The environmental gradient of the study area is divided into groups; within each group an (ideally) equal number of samples is collected at random. This approach often yields better model results than a regular or random sampling design, since it controls for a gradual distribution of samples along the environmental gradient.
- (iii) *Gradsect*: In this sampling strategy, the groups are unevenly distributed along the environmental gradient in a few transects scattered across the study area, allowing for random sampling while minimizing time and costs.

In recent years, the availability of open source environmental data sets in many different formats and resolutions has increased considerably, providing very valuable information for habitat suitability modelling. Such data sets are derived from diverse sources such as remotely sensed imagery and raster maps.

4. IMPLEMENTATION

4.1. Model formulation and assumptions

The statistical formulation of an HSM requires the selection of a modelling approach and technique suited to the particular type of response variable (i.e. species' occurrence data) and its probability distribution in order to correctly estimate the model coefficients (Guisan and Zimmermann 2000; Guisan and Thuiller 2005). Among the currently available modelling options for HSMs, we focus on the multiple regression approach and the family of generalised linear models (GLMs), which are frequently applied in species conservation and implemented in numerous statistical software packages (Schröder et al. 2009).

GLMs represent a form of linear regression models that are able to cope with non-normal distributions of the response variable such as binominal or Poisson (Guisan and Zimmermann 2000). In the GLM, a single predictor variable or a set of predictors, a linear predictor, is related to the response variable through a link-function that allows transformation to linearity and prediction within the range of the observations (Guisan and Zimmermann 2000). Before drawing conclusions from the analysis, it is important to check the validity of the linearity assumption implicit in the GLM definition (Crawley 2002; Guisan et al. 2002; Luoto et al. 2005), as well as to test the statistical independence of the observations. Spatial autocorrelation is often encountered when the raw data show a dependence structure; this can easily be quantified by checking the model residuals (Lichtenstein et al. 2002; Fortin and Dale 2005; Dormann 2007; Kühn 2007).

4.2. Model calibration and selection

To allow for high model accuracy and predictive power, the number of predictor variables in the final HSM should not exceed $n/10$, where n is the number of observations in the smallest represented category (Harrell et al. 1996). In addition, predictors must be independent of each other/non-correlated. There are many approaches to detect collinearity (i.e. the existence of correlation) between predictors and to identify those which are to be dropped from the HSM (Zuur et al. 2010): (i) correlation coefficients (e.g. Spearman's rank), (ii) a principal components analysis (PCA), and (iii) the variance inflation factor (VIF) that quantifies how much of the variance of the estimated model coefficients is influenced by collinearity.

Minimal adequate HSMs can be determined by backward and forward stepwise model selection procedures (Harrell Jr. 2001) based on the Akaike's information criterion (AIC) (Akaike 1978) and the Bayesian information criterion (BIC) (Schwarz 1978) that favour a model that has good fit to the data but few parameters. From the set of candidate HSMs, the model with the lowest AIC and BIC values is the one to be preferred.

4.3. Model performance

Besides testing the statistical significance of an HSM, assessing its predictive performance is necessary to characterize how well or poorly the model achieves the study goals. Some measures of performance assess the goodness of fit to the observed presences and absences, allowing comparison between models that include different sets of predictor variables, such as Nagelkerke's coefficient of determination (Nagelkerke 1991). Other measures of performance indicate the model's ability to discriminate and rank presences and absences correctly:

- (i) *Threshold-dependent measures*, which require the selection of a threshold of occurrence (i.e. the value at or above which an HSM is deemed to predict presence of the species), such as sensitivity (the probability that the model will correctly classify a presence), specificity (the probability that the model will correctly classify an absence), and Cohen's Kappa statistic (combining both probabilities in one parameter), and
- (ii) *Threshold-independent measures*, such as the area under the curve (AUC) of the receiver operating characteristic (ROC) plot, a scatter plot of sensitivity *versus* the term 1-specificity for all possible classification thresholds between 0 and 1 (Hanley and McNeil 1982; Zweig and Campbell 1993; Fielding and Bell 1997). A model is considered to discriminate better than chance if the AUC is higher than 0.5, and higher than 0.7 is actually allowing valid predictions.

Generally, the HSM for which the deviance reduction is maximal is considered the best fitted model and is used to predict the distribution of species (Guisan and Zimmermann 2000; Reineking and Schröder 2003).

4.4. Model evaluation

Two main approaches are used to evaluate the accuracy of model predictions and field observations (i.e. accuracy of predictions or model predictive power) (Guisan and Zimmermann 2000):

- (i) *Internal model evaluation or verification*, using a single data set to calibrate and evaluate the model. In this case, model evaluation techniques include re-sampling and data splitting methods such as jackknife (JK), bootstrap and k-fold cross-validation (CV) (Verbyla and Litvaitis 1989; Chatfield 1995; Manel et al. 1999), and
- (ii) *External model evaluation or validation*; using two independent data sets from different locations or times, one for calibrating (i.e. calibration or training data set) and the other for evaluating the model (i.e. evaluation or testing data set).

For both model evaluation approaches, the above-mentioned threshold-dependent and -independent measures can be used to compare predictions to observations and assess model predictive accuracy.

4.5. Model transferability

In conservation planning, it is of particular interest whether an HSM calibrated in one region (or time period) can be applied to another region (or time period). Successful model transferability (i.e. cross-applicability or generality) implies that a final HSM can be used to predict species' occurrences beyond the study area (or time) the model was fitted for (Binzenhöfer et al. 2005; Randin et al. 2006; Vanreusel et al. 2007). Therefore, transferable HSMs can be used to inform conservation managers regarding optimal reserve design and species' reintroduction success. Transferability can be evaluated with the above mentioned threshold-dependent and -independent measures, and, for example, is often assumed to fail for models with AUC values lower than 0.7 (Randin et al. 2006). Besides spatial and temporal transferability, the applicability of HSMs across all the stages of the life cycle (e.g. larvae and adults of butterflies) is important when designing species' conservation guidelines (Radchuk et al. 2013).

5. APPLICATIONS AND LIMITATIONS

Conservation management policies rely on the ability to predict the spatial distribution of species, both under current and future environmental scenarios. Numerous examples exist which confirm the validity of the habitat suitability modelling approach based on GLMs as a useful tool to obtain basic knowledge about species' habitat requirements and to understand species-environment relationships and representing the first step towards anticipating future impacts of environmental changes on species' distributions (Schröder et al. 2009). As such, HSMs provide the predictive basis for a wide range of applications in conservation planning (e.g. location of habitats suitable for colonization and reintroduction, estimation of extinction risks, prioritization of conservation areas and design of reserve networks). However, since HSMs are based on the species-environment equilibrium assumption, models may produce unreliable predictions in many situations in which species do not occupy all the suitable habitats (e.g. invasive species, species threatened by human perturbation, species subjected to dispersal limitations). In the case of butterfly species, the information yielded by HSMs on larval habitat requirements and adult responses to habitat perturbation has led to the estimation of the effects of climate change and to sound management recommendations aimed at protecting endangered species such as *Lycaena helle* (Turlure et al. 2009, 2010; Goffart et al. 2010).

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Resource-based habitat use in *Lycaena helle*: Significance of a functional, ecological niche-oriented approach

Camille Turlure^{1*}, Hans Van Dyck¹, Philippe Goffart² & Nicolas Schtickzelle¹

¹Université catholique de Louvain, Earth and Life Institute, Biodiversity Research Centre, B-1348 Louvain-la-Neuve ²Département de l'Étude du Milieu naturel et agricole (DEMna), Service Public de Wallonie (SPW), B-5030 Gembloux

*Corresponding author: Camille Turlure, Université catholique de Louvain, Earth and Life Institute, Biodiversity Research Centre, Place Croix du Sud, 4, B-1348 Louvain-la-Neuve, E-Mail: camille.turlure@uclouvain.be; turlure_camille@hotmail.com

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Abstract

We adopted a resource-based habitat approach to better understand the distribution and conservation of the Violet Copper butterfly, the focal species of this book. The significance of this organism-centered, behavioural approach of habitat to understand and conserve butterflies, instead of using general vegetation types as surrogates of a species' habitat, has recently been advocated. This approach aims for integrating the key functional relationships between the organism and the essential consumables and conditions in the environment focusing on both adult and larval stages. Thus, we summarized our knowledge on the resource-based habitat-use in *L. helle* based on field data from the Belgian Ardenne region. We included demographic and behavioural data and models explaining the abundance of males and females, the egg distribution and the survival from the egg stage until the third larval instar. Finally, we discussed the new insights on habitat-use of *L. helle* across its life cycle and the significance of these findings and the resource-based habitat concept for the conservation of *L. helle* in Belgium and elsewhere.

RESOURCE-BASED HABITAT DEFINITION

Spatial data at the landscape level like remote sensing data and digital land cover maps are readily available nowadays. Therefore, a growing number of studies on species distribution make use of such land cover data. Such applications can be interesting for policy making on spatial conservation planning as they allow addressing several conservation issues at the broader spatial scale (e.g. conservation priority areas; Maes

et al. 2005). However, if it is taken for granted that such general vegetation units represent the functional resolution at which organisms deal with their environment, it may confound our understanding of organism–environment interactions and what it means for conservation (Hall et al. 1997; Dennis et al. 2006). Vegetation maps show structural variation as it is visually perceived by the human observer, but this may deviate considerably from what is the functional habitat through the eyes (or other sensory organs) of a focal species. The identification of specific, fine-scaled habitat variables within and across different vegetation types may be necessary to understand the presence, abundance and persistence of particular species, including butterflies (Vanreusel and Van Dyck 2007; Turlure et al. 2009).

The issue of recognizing functional habitat on the ground is of particular interest for conservation programs in anthropogenic landscapes. In these landscapes there is a particular risk of losing specific resources from certain vegetation types, even without losing the vegetation *per se*. Intensification of land-use resulted in the loss and fragmentation of natural habitats, but it also applies to so-called semi-natural habitats. The latter vegetation types have evolved from the interaction between traditional human management (e.g. small scale mowing, sod-cutting or grazing) and the responses from naturally occurring species over several centuries (or longer). In regions with a long history of human settlement and with hardly any natural vegetation being left, semi-natural vegetation has become the major focus of conservation concern. Well-established examples include several types of unimproved grasslands, coppiced woodlands and heathlands. Butterflies in particular have been successful in colonizing and adapting to such environments (Thomas 1993). A global analysis of biotope-use and status trends in the 576 European butterflies, including threatened species, showed that the most important biotopes in terms of species diversity (and rarity) are man-made or man-influenced communities, notably different types of grassland and heath or scrub communities (van Swaay et al. 2006).

In our study, we adopted a resource-based habitat approach to better understand the distribution and conservation of the focal species of this book – *Lycaena helle*. It has been noticed since long that *L. helle* may be absent from vegetation units that would generally fit its ecological profile, including the presence of the host plant. The significance of this organism-centered, behavioural approach of habitat to understand and conserve butterflies (and other organisms), instead of using general vegetation types as surrogates of a species' habitat, has recently been advocated (Dennis et al. 2003; Shreeve et al. 2004). Several recent studies have adopted this resource-based habitat concept in butterflies (Fred et al. 2006; Grundel and Pavlovic 2007; Turlure et al. 2010a). For a detailed discussion, we refer to the textbook by Dennis (2010). This approach challenges the widely applied bipolar view of landscapes with sharply defined patches of presumed suitable habitat in a matrix of unsuitable habitat as it views the landscape as a continuum of overlapping resource distributions. This approach aims for integrating the key functional relationships between the organism and the essential consumables and conditions in the environment focusing on both adult and larval stages (Dennis et al. 2003). So, the resource-based habitat concept

reconnects the definition of habitat to the concept of the ecological niche. For butterflies, resources include host plants and nectar plants under suitable microclimatic conditions, but also other specific conditions and structures for mate-locating and roosting sites, among others (Dennis et al. 2006). Microclimatic conditions are of particular significance to butterfly habitat use, in all stages (Dennis 1993). It is the interaction between the regional climate and the local environmental conditions (topography and vegetation structures) that generates the thermal area in which butterflies operate (Suggitt et al. 2011; Turlure et al. 2011). In semi-natural vegetation units, the presence, abundance and spatial configuration of different ecological resources and conditions are directly affected by the type, frequency and scale of management (Dolek and Geyer 1997; Bergman 2001; Dolek, and Geyer 2002), but also by other sources of disturbance including pollution (e.g., atmospheric nitrogen deposition; WallisDeVries and Van Swaay 2006).

Here, we present and summarize our knowledge on the resource-based habitat-use in *L. helle* based on largely unpublished field data from the Belgian Ardenne region. After a brief introduction to the study system in Belgium, we will present and discuss the following ecological and behavioural aspects of habitat-use: (i) demographic information based on a mark-release-recapture study, (ii) models explaining the abundance of males and females relative to vegetation characteristics, (iii) behavioural data on adults focusing on thermoregulation, (iv) data on adult movements, (v) models explaining egg distribution, and (vi) models explaining survival rates from the egg stage until the third larval instar under natural conditions. We will discuss new insights on habitat-use of *L. helle* along its life cycle and the significance of these findings and the resource-based habitat concept for the conservation of *L. helle* in Belgium and elsewhere.

RESOURCE-BASED HABITAT FOR *LYCAENA HELLE* IN THE BELGIAN ARDENNE

Lycaena helle is a boreo-montane species widely distributed across central and northern Europe, but restricted to few localities where it can be locally abundant. In Scandinavia, the species use sorrels as host plants (mainly *Rumex acetosa* and *R. acetosella*) and knotgrass further in the North (*Polygonum viviparum*). The host plant species used in Mongolia is not yet known (Chuluunbaatar et al. 2009). In western Europe, like in Germany, in France and in Belgium, the species is declining and restricted to mountain regions where *Polygonum bistorta* (the only host plant used in this region) occurs in high abundance (Bachelard and Descimon 1999; Fischer et al. 1999; Fichet et al. 2008). In this region, the typical habitat has been described as sunny and wet openings near forest or on drier terrain with abundant flowers, under sheltered and warm conditions. In northern Mongolia, it was described as wet mesophile grasslands and moist clearings in forests (Chuluunbaatar et al. 2009). Most studies focused however on adult distribution across and within habitat patches; factors affecting the presence, abundance and survival of the other life stages have often been neglected.

Study site

Field data were collected in the Fange de Pisserotte nature reserve, a 56 ha peat bog located on the southern side of the Plateau des Tailles (S-Belgium: 50°13'N, 5°47'E), and surrounded by birch and willow as well as by spruce plantations. Within this peat bog, we selected 35 zones based on their general vegetation aspect (14 ha in total; Fig. 1).

In 2005, ten randomly placed vegetation samples were recorded with a 1 m² grid to measure the abundance of each plant species on a 0 to 25 scale in each zone. The first composite axis ($DCA1_z$; eigenvalue = 40.1%) of a Detrended Correspondence Analysis (Canoco software Version 4.5; Ter Braak and Smilauer 2002) on the plant species abundances of the 350 samples was used as a vegetation type index. Lower and negative values of $DCA1_z$ corresponded to wet meadows (with *Deschampsia cespitosa*), rushes (with *Juncus acutiflorus*) and fen grasslands (with *Cirsium palustre*, *Angelica sylvestris* and *Rumex acetosa*), whereas higher positive values were associated with heathlands (with *Calluna vulgaris* and *Vaccinium myrtillus*) and swamps (with *Narthecium ossifragum* and *Vaccinium oxycoccus*; Fig. 2). Most of the nectar plants used by *L. helle* were more abundant for lower values of $DCA1_z$. Zones were also classified in three classes according to their *EDGE* structure: no edge, some nearby trees, surrounded by trees. The host plant *P. bistorta* occurred in 23 zones covering 17,490 m² in total. *BISTORT* abundance in each zone was computed as the sum of the *P. bistorta* abundance in the ten vegetation samples.

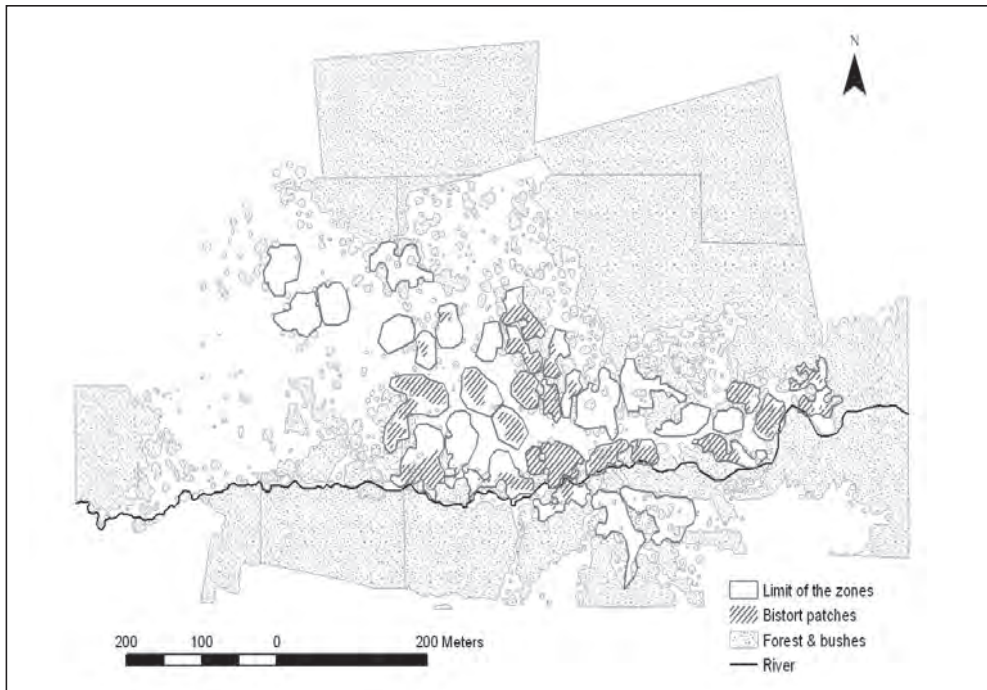


Figure 1: Map of the study area. Grey line: limit of the 35 zones. Dashed area: *P. bistorta* patches.

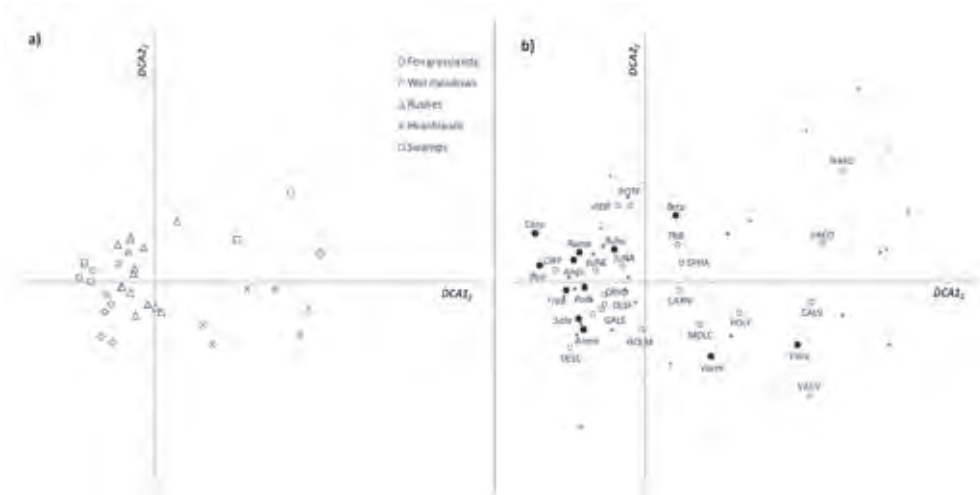


Figure 2: Graphical representation of the Detrended Correspondence Analysis at the zone level. a) Location of the 35 zones in the bidimensional space made of the two first composite axis, $DCA1_z$ and $DCA2_z$. Symbols represent the five vegetation types. b) Position of each plant species in the same bidimensional space. Big circles: plants that contributed the most to the axis formation. Grey dots: other plant species. Black circles: nectar resources used by *L. helle* adults. Abbreviations: ANEN *Anemone nemorosa*, ANGS *Angelica sylvestris*, BETP *Betula pubescens*, CALV *Calluna vulgaris*, CARP *Cardamines pratensis*, CARN *Carex nigra*, CIRP *Cirsium palustre*, DESC *Deschampsia cespitosa*, DESF *D. flexuosa*, DRYO *Dryopteris* sp., FRAA *Frangula alnus*, GALS *Galium saxatile*, HOLM *Holcus mollis*, JUNA *Juncus acutiflorus*, JUNE *J. effusus*, MOLC *Molinia caerulea*, NARO *Narthecium ossifragum*, POLB *Polygonum bistorta*, POLY *Polytrichum* sp., POTP *Potentilla palustris*, RUBU *Rubus* sp., RUMA *Rumex acetosa*, SALA *Salix aurita*, SPHA *Sphagnum* sp., TRIE *Trientalis europaea*, VACM *Vaccinium myrtillus*, VACO *V. oxycoccus*, VACU *V. uliginosum*, VACV *V. vitis-idaea*, VALR *Valeriana repens*, VIOP *Viola palustris*.

Adult stage

a) Demographic parameters

During the flight period of 2005, we monitored population size using a Mark-Release-Recapture study (MRR) (further details on this method are given in Box II). The study site was visited daily under suitable weather conditions (i.e. no strong wind, no or few clouds, and air temperature $> 15^{\circ}\text{C}$). Adults were individually marked and immediately released. We recorded date, location (i.e. one of the 35 predefined zones), marking code and sex at each (re)capture.

Demographic parameters were inferred from these MRR data using Jolly-Seber open population models implemented in the MARK program (White and Burnham 1999). Estimated population size was larger for females than for males ($927 \text{ females} \pm$

237 vs. 831 males \pm 171; Given are means \pm 95% confidence intervals). Daily survival decreased over time (from May 15 to June 29) for both sexes and was lower for females than for males (86% vs. 91% on average, respectively). Average catchability was low for both sexes compared with studies on the same species in other areas (ranging from 6 to 71% in Fischer et al. 1999, 21% in Chuluunbaatar et al. 2009) and with other butterfly species (*Lycaena virgaureae*: 29% in Schneider et al. 2003; *Boloria eunomia*: 24 to 46% in Schtickzelle et al. 2002; *Euphydryas aurinia*: 26 to 37% in Junker and Schmitt 2010). It was lower for females than for males (4.5% vs. 6.0%, respectively).

b) Spatial distribution of adults

Male and female densities (dM and dF , respectively) in each zone were calculated as the total number of (re)capture events per unit of area. As catchability was lower for females, female densities were weighed by the ratio of catchability between sexes. Adult density in each of the 35 zones ranged from 0–27 and 0–39 for females and males, respectively. Results of generalized regression models (Table 1a for model selection among all possible combinations of the following variables: SEX , $BISTORT$, $DCA1_z$, $DCA1_z^2$, $EDGE$, $SEX*BISTORT$, $SEX*DCA1_z$, $SEX*DCA1_z^2$, $SEX*EDGE$; Table 2a for parameter estimates) indicated that density for both sexes was (i) higher in zones with higher $BISTORT$ abundance, (ii) higher in optimal vegetation types (wet meadows, fen grasslands and rushes; negative effect of $DCA1_z$ and its square value), and (iii) in zones surrounded by more tree edges. The $EDGE$ structure effect was more pronounced in males than in females.

c) Adult time budget and behaviour

In 2006, we individually tracked a random sample of 34 males and 32 females, during 5 min each under suitable weather conditions, and recorded their behaviour: thermoregulating (i.e. resting, dorsal basking and hindwing rubbing), nectar feeding (and the flower species used), flight, interaction with other organisms during flights and oviposition (Jones 1977; Stanton 1982). By far, the two sexes spent the majority of their active time budget basking to thermoregulate (Fig. 3), with higher total time ($F_{1,65} = 8.67$, $P = 0.004$) and mean duration of individual bouts ($F_{1,425} = 3.52$, $P = 0.061$) in females than in males. The proportion of time allocated to feeding was not significantly different between both sexes ($F_{1,65} = 0.81$, $P = 0.373$), but females had on average longer feeding bouts than males ($F_{1,54} = 4.27$, $P = 0.044$). Females spent proportionally less time in flight ($F_{1,65} = 20.39$, $P < 0.001$) with shorter mean duration of flight bouts ($F_{1,264} = 6.47$, $P = 0.011$) than males did.

Thermoregulation occurred on 20 different substrates, mostly on nectar plants, but also on trees (in 16% of the cases for males vs. 5% for females). 77% of the interactions engaged by males were with conspecifics, the others were with Diptera, Hymenoptera

Table 1: Modeling a) adult density, b) emigration probability, c) dispersal probability, d) egg number (sampling effort was added as a covariate), e) egg hatching and f) caterpillar survival using generalized linear models with AICc model selection (Anderson 2008). The list of models is restricted to supported models and “intercept only” models. K: number of estimated parameters, LogL: log-likelihood, ΔAICc : AICc difference with the lowest AICc model. Selected best model is in bold. For c) dispersal probability was computed as the probability to disperse farther than a certain distance.

	Y	Error distribution	Link function	Explanatory variables in the model	K	LogL	AICc	ΔAICc
a)	Adult density	Poisson	Log	Sex + Bistort + DCA1_Z + DCA1_Z^2 + Edge + Sex*Edge	9	-227.80	476.60	
				Sex + Bistort + DCA1_Z + DCA1_Z^2 + Edge + Sex*Bistort + Sex*Edge	10	-227.67	479.06	2.47
				...				
				Intercept only	1	-441.94	885.93	409.33
b)	Emigration probability	Binomial	Logit	Sex ratio + Adult density	3	-83.93	174.05	
				Sex + Sex ratio + Adult density	4	-83.53	175.37	1.32
				Adult density	2	-85.93	175.95	1.90
				...				
c)	Dispersal probability	Normal	Identity	Intercept only	1	-90.44	182.90	8.85
				ln(distance)	3	64.89	-123.34	
				ln(distance) + Sex	4	65.21	-121.67	1.67
				...				
d)	Egg number	Poisson	Log	Intercept only	2	34.10	-63.99	59.35
				Sampling effort + dM + dF + DCA1_Z + DCA1_Z^2	6	-198.62	412.25	
				Sampling effort + dM + DCA1_Z + DCA1_Z^2	5	-200.29	412.66	0.41
				Sampling effort + dM + Host + DCA1_Z + DCA1_Z^2	6	-198.90	412.80	0.56
				Sampling effort + dM + dF + Host + DCA1_Z + DCA1_Z^2	7	-197.94	414.02	1.77
				...				
e)	Egg hatching	Binomial	Logit	Sampling effort only	2	-253.86	512.09	99.85
				DCA1_E + RatioH + L	4	-42.82	93.96	
				DCA2_E + RatioH	3	-44.00	94.18	0.22
				RatioH	2	-45.16	94.41	0.45
				DCA2_E + RatioH + H	4	-43.16	94.63	0.67
				L	2	-45.31	94.71	0.75
				DCA2_E + DCA2_E^2 + RatioH	4	-43.22	94.76	0.80
f)	Caterpillar survival	Binomial	Logit	Intercept only	1	-46.37	94.77	0.81
				...				
				L + H	3	-45.63	97.48	
				L + T + H	4	-45.37	99.10	1.61
				Host + L + H	4	-45.37	99.11	1.63
				DCA2_E + L + H	4	-45.51	99.39	1.90
				...				
				Intercept only	1	-52.94	107.92	10.44

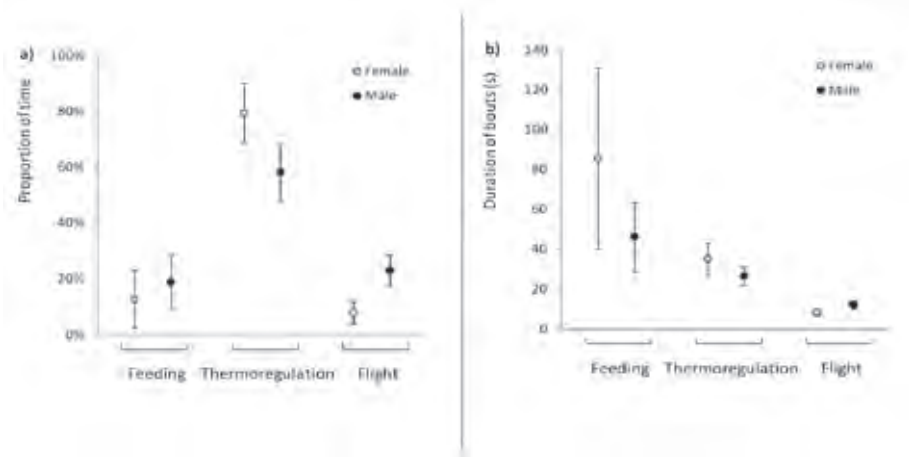


Figure 3: Proportion of time (a) and duration of bouts (b) for the three behaviour types (feeding, thermoregulation and flight). Grey dots: females. Black dots: males. Given are means \pm 95% confidence intervals.

and other butterfly species. Oviposition behaviour including both pre-alightment and egg-laying behaviours covered on average 7% of the active time budget of females. In 52% of the cases, typical oviposition pre-alightment behaviour at a host plant was not followed by egg-laying, but instead the female flew away. The duration of the pre-alightment behaviour did not differ significantly whether it was or not followed by egg-laying (ANOVA: $F_{1,39} = 1.28$, $P = 0.26$; without egg-laying: 9.9 s vs. with egg-laying = 13.4 s).

d) Adult movements

From the MRR data, we extracted movement data using pairs of successive (re) captures of any given marked individual. Each pair gave two types of information: (i) emigration (recapture in a zone distinct from the initial marking zone) vs. no emigration (recapture in the same zone), and (ii) distance travelled (between zone centres). Out of the 136 pairs of successive (re)captures, 83 implied emigration and movement between two zones. Distance travelled in these movements was on average 108 m, with a maximum of 335 m for males and 522 m for females. Distances between zones were on average 242 m (min = 27 m; max = 728 m).

Emigration probability was similar for both sexes and increased with lower local *ADULT DENSITY* and female biased *SEX-RATIO* in the departure zone (*SEX-RATIO* was female density / total adult density) (Table 1b for model selection; Table 2b for parameter estimates). We did not detect any interaction effect with sex. Dispersal probability, modelled by an inverse-power function, showed a very strong decrease with *DISTANCE*, but did not differ between sexes (Table 1c for model selection; Table 2c for parameter estimates; see Schtickzelle et al. 2012 for details on dispersal kernel estimation).

Table 2: Factors affecting a) adult density, b) emigration probability, c) dispersal probability, d) egg number (sampling effort was added as a covariate) and e) caterpillar survival (estimated using the best model from Table 1). For categorical variables, the estimate expresses the difference with the reference level (fixed to zero).

Y	Parameter			Estimate	Standard error
a) Adult density	Intercept			1.402	0.203
	Sex	Female		-0.211	0.188
		Male		0.000	.
	Bistort abundance			0.006	0.001
	DCA1 _Z			-0.658	0.127
	DCA1 _Z ²			-0.153	0.081
	Edge	No edge		-2.989	0.822
		Some nearby trees		-0.230	0.130
		Surrounded by trees		0.000	.
	Edge * Sex	No edge	Female	1.441	0.681
			Male	0.000	.
		Some nearby trees	Female	0.100	0.151
			Male	0.000	.
		Surrounded by trees	Female	0.000	.
			Male	0.000	.
b) Emigration probability	Intercept			0.313	0.760
	Sex ratio			2.663	1.389
	Adult density			-0.031	0.013
c) Dispersal probability	Intercept			0.695	0.048
	ln (distance)			-0.106	0.010
d) Egg number	Intercept			2.519	0.091
	Sampling effort (covariate)			0.004	0.000
	Male density			-0.032	0.007
	Female density			0.019	0.010
	DCA1 _E			-0.346	0.084
	DCA1 _E ²			-0.272	0.066
e) Caterpillar survival	Intercept			-1.719	8.369
	L			-2.334	1.033
	H			2.970	0.916

Egg and caterpillar stages

In 2005 (starting on May 22nd), we searched for eggs in all host plant patches (time spent searching was 77 hours in total by one to three persons simultaneously). For each of the 692 eggs found, we recorded: (i) the position on the host plant (i.e., distances to the border and to the basis of the leaf), (ii) the size of the host plant leaf (i.e., the maximal length and width), (iii) the relative height of the egg-laying site compared with the height of the surrounding vegetation (*RatioH*), and (iv) the abundance of each plant species within a one 1 m² plot (0 to 25 scale) for each of the 355 locations where the eggs were found. From all these samples, we extracted the *Host* abundance and summarized the

vegetation composition by the two first composite axes ($DCA1_E$ and $DCA2_E$, cumulative eigenvalue = 45.9%; Fig. 4) of a Detrended Correspondence Analysis on the abundance of the plant species. Local conditions of moisture (H), light intensity (L) and temperature (T) were inferred for each of the 355 vegetation samples based on the abundance-weighted mean of Ellenberg indicator values of plant species (Ellenberg 1992).

All eggs were laid on the underside of the host plant leaves. The size of the chosen leaves varied considerably, ranging from 4.0 to 19.5 cm in length and from 2.4 to 9.5 cm in width. The pattern of egg deposition was highly constant; the egg was on average deposited at 1.3 cm from the border of the leaf (between 0 and 3.75 cm) and somewhat less frequently at the tip compared with the middle and basal parts of the leaf (tip: 20%, middle: 44%, base: 36%).

More eggs were found in zones (i) of optimal vegetation types (i.e., wet meadow, fen grassland and rush vegetation types; lower values of $DCA1_Z$ and $DCA1_Z^2$), (ii) with higher female density dF , and (iii) with lower male density dM (Table 1d for model selection; Table 2d for parameter estimates). We did not detect any effect of $EDGE$ structure or $HOST$ abundance.

A random sample of 130 eggs was monitored until July 8th to study egg hatching and caterpillar survival. We checked the eggs every two days until hatching and then we searched for the small caterpillars under the *P. bistorta* leaf on which the egg was depos-

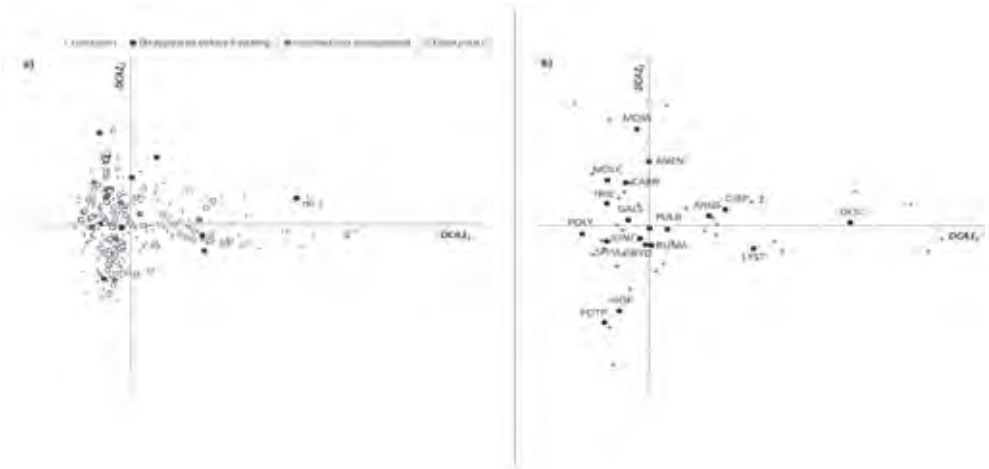


Figure 4: Graphical representation of the Detrended Correspondence Analysis at the egg-laying site level. a) Location of the 692 eggs in the bidimensional space made of the two first composite axis, $DCA1_E$ and $DCA2_E$. Symbols represent the status of the eggs as described in the text. b) Position of each plant species in the same bidimensional space. Big circles: plants that contributed the most to the axis formation. Grey dots: other plant species. Abbreviations: ANEN *Anemone nemorosa*, ANG *Angelica sylvestris*, CARN *Carex nigra*, CIRP *Cirsium palustre*, DESC *Deschampsia cespitosa*, DESF *D. flexuosa*, DRYO *Dryopteris* sp., GAL *Galium saxatile*, JUNA *Juncus acutiflorus*, LYST *Lysimachia thyrsiflora*, MOLC *Molinia caerulea*, MOSS Moss species, POLB *Polygonum bistorta*, POLY *Polytrichum* sp., POTP *Potentilla palustris*, RUMA *Rumex acetosa*, SPHA *Sphagnum* sp., TRIE *Trientalis europaea*, VIOP *Viola palustris*

ited and under the adjacent ones in the case it has moved (15 cm apart from the egg). Indeed, *L. helle* caterpillars remain under *P. bistorta* leaves until the leaves are completely eaten, and next they move to closer leaves till pupation. Each egg was classified in one of these three categories: eggs that disappeared before hatching, eggs that hatched but the small caterpillar disappeared a few days after, or eggs whose caterpillar reached the third instar. Only 12% of the eggs did not hatch and egg hatching was not explained by any of the tested variables (i.e. $DCA1_E$, $DCA1_E^2$, $DCA2_E$, $DCA2_E^2$, *Host* abundance, *L*, *H*, *T* and *RatioH*; See Table 1e for model selection). 82% of the caterpillars survived until the third instar. We tested the same set of explanatory variables as listed previously on caterpillar survival. It increased only in more humid (higher *H* value) and darker (lower *L* value) conditions (Table 1f for model selection; Table 2e for parameter estimates).

NEW INSIGHTS ON HABITAT USE ACROSS THE LIFE CYCLE

What actually is the habitat of the adults?

Not surprisingly, adult density increased with host plant abundance. It was expected since *P. bistorta* is in the same time (i) the single host plant used by caterpillars and egg-laying females in Belgium and (ii) a nectar resource frequently used by adults. Additionally, adult density was found higher in some vegetation types because it provided an additional set of nectar resources. Indeed, wet meadows and especially fen grasslands offer the highest density and variety of nectar resources used by the species. Since the flight period starts in early May, when *P. bistorta* is not yet blooming, adults may rely on other plant species to feed. Chuluunbaatar et al. (2009) also described an opportunistic use of nectar resources for *L. helle* in Mongolia, with this use changing along the flight period. This strong association with particular vegetation types was already underlined; Fischer et al. (1999) described the typical habitat of *L. helle* as moist meadows with abundant knotgrass, *Deschampsia cespitosa*-*Polygonum bistorta*-association, fallow stage of unimproved moist grassland.

The presence and structure of edges as an important component of *L. helle* habitat was already suggested in several studies. For example, Bauerfeind et al. (2009) found that the presence of trees and scrubs in occupied patches was higher compared with vacant patches in a German meta-population. The presence of trees and edges may be beneficial, or even necessary, for several reasons.

Firstly, many tree and bush species, such as *Salix aurita*, *Frangula alnus* and *Sorbus aucuparia* in the Pisserotte site (but also *Prunus spinosa*, *Crataegus monogyna*, *Viburnum opulus* on other sites) are used as nectar resources, especially at the beginning of the flying period when no or few herbaceous plant species are yet flowering. Nectar feeding has been shown to increase longevity and survival in several butterfly species (e.g. *Lycaena tityrus*, Karl and Fischer 2009; *Araschnia levana*, Mevi-Schutz and Erhardt 2005).

Secondly, edges may provide suitable microclimatic conditions. As suggested by Dover et al. (1997), it can shelter against the wind. Moreover, the thermal profile of edges

may be described as follow: during the night and at the beginning of the day, temperature decreased from the top to the bottom of edges, whereas during the day, temperature is higher in the middle position compared with both the ground and top position (Turlure, unpublished data; Fig. 5). Activity of *L. helle* is strongly correlated with solar radiation and we observed the individuals to spend most of their time budget thermoregulating. Basking close to or in the edges during the day may hence be thermally interesting or even necessary for a late spring flying species (i.e., when daily temperatures are relatively low and the impact of convective cooling rather strong). The reverse situation was observed for *Lycaena hermes*; bushes providing shade during hot conditions (Marschalek and Klein 2010). This is also in line with behavioural observations of adults reaching the top of trees (i.e., where the temperature is higher than near the ground during the night; Fig. 5), at the end of the day to spend the night (Goffart, unpublished data). This strategy probably allows individuals (males in particular) to start their activity earlier in the morning and hence to occupy a territory more rapidly.

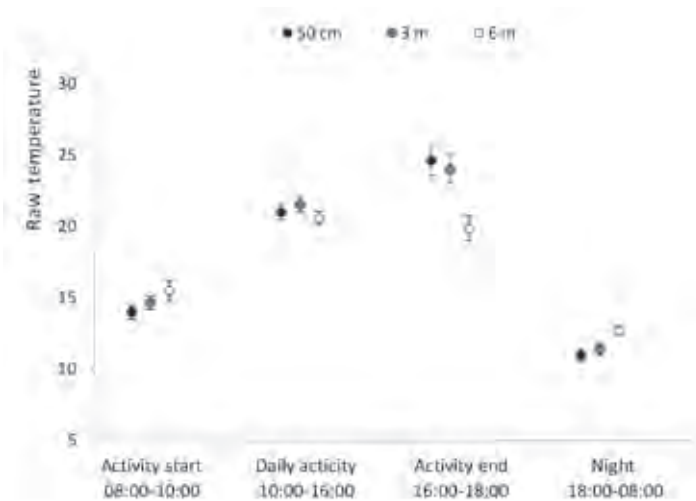


Figure 5: Thermal profile of edges according to the period of the day and the height at which the temperature was recorded. Given are means \pm 95% confidence intervals. During the night and at the beginning of *L. helle* activity, temperature decreased from the top of edges to the ground. Temperature was slightly higher for the middle position for 10:00-16:00 time period, and higher in the ground and middle positions compared with the top position at the end of *L. helle* activity. Note: In order to investigate the thermal profile of edges, we placed temperature recorders (temperature recorded every five minutes; Onset HOBO UA-002-64; www.onsetcomp.com) at three height positions (at 0.5, 3 and 6 m from the ground, later referred to as ground, middle and top positions, respectively) on edges during the flight period. The datasets were split into four period of the day: (i) from 8:00 to 10:00, period at which adults start to be active, (ii) from 10:00 to 16:00, the main activity period, (iii) from 16:00 to 18:00, period at which adults stop to be active and (iv) the late afternoon and night period without activity (from 18:00 to 8:00).

Finally, sunny edges are also used as encounter sites, where males perch and defend territories, waiting for females, such as observed for *Lycaena arota* (Scott 1973). Under high densities of individuals, like in Pisserotte, males may aggregate on landmarks, as already described in *Coenonympha pamphilus* (Wickman et al. 1995). This probably explains why the edge structure effect on adult density was more pronounced in males compared with females.

What actually is the habitat of the eggs and caterpillars?

In the Pisserotte site, we observed that more eggs were deposited, as expected, in zones with a higher density of females, but also in zones with a lower density of males. This may be related to the female harassment by territorial males as shown in *Lycaena hippothoe* (Turlure and Van Dyck 2009). Surprisingly, no effect of host plant abundance was detected. In *L. helle*, eggs are deposited one by one, and few egg-laying sites supported more than one egg; females maybe recognise conspecific eggs and avoid laying in occupied places such as suggested by Dempster (1992) or Vasconcellos Neto and Monteiro (1993) for other species. This behaviour results in a single caterpillar per egg-laying site, no intra-specific food competition and hence no need for a high local abundance of the host plant. In the laboratory, one individual can feed and achieve the pupal stage with only four to six *P. bistorta* leaves. In the case of *Boloria eunomia* and *Argynnis niobe*, host plant abundance (among other factors) best explained the pattern of egg and caterpillar presence, but up to around 20 eggs or six caterpillars can be found together (Goffart and Waeyenbergh 1994; Salz and Fartmann 2009; Turlure, personal observation).

The observed hatching rate of the subsample of eggs in the field was high (88%). All the eggs were found on the underside of the host leave, probably conferring protection from desiccation risk due to direct solar radiation or wind. Moreover, duration of the egg stage is relatively short in *L. helle*; in the laboratory, eggs hatched after six to 13 days. We probably failed to highlight any effect of the tested parameters on egg hatching for these reasons. This was obviously not an artefact of the random subsample (but see Fig. 4). In other butterfly species, hatching rate was mostly influenced by temperature, negatively in *L. hippothoe* (Fischer and Fiedler 2001), positively in *B. eunomia* (Radchuk et al. 2013). Furthermore, Fischer et al. (2003) showed that the larger eggs produced by females of the (sub)tropical *Bicyclus anynana* at a lower temperature had a higher hatching success.

Caterpillars reached the 3rd instar after ten to 18 days, and survival until this stage was high (82%). For comparison, it was only 16% under suitable natural conditions for *Lycaena xanthoides* (Severns et al. 2006). Females were relatively choosy; 52% of the pre-alightments were not followed by egg-laying. Females may confer higher survival to their offspring by choosing qualitatively suitable egg-laying sites to support larval growth and development (Doak et al. 2006). This does not only imply finding the suitable host plant, but also the suitable microclimatic conditions. Survival was affected by microclimatic conditions only, sufficient humidity and shadow conferring higher survival.

Understanding the mechanisms that explain the higher survival rate under such conditions surely deserves appropriate experiments under controlled conditions. However, we can postulate that these conditions are suitable for host plant development and hence caterpillar feeding and survival (Bernays and Chapman 1994).

In the present state, we surely lack important data on the pupal stage. The pupa probably is the critical stage in the *L. helle* life cycle since it overwinters and lasts around 300 days. Finding pupae in the field is nearly an unfeasible task; in 3 years of prospection, we only observed 20 pupae in the field. Nevertheless, defining habitat requirements and testing the influence of environmental parameters on pupal survival would be conceivable using reared individuals and greenhouse experiments.

Neither egg and caterpillar parasitism nor caterpillar cannibalism (as observed on other *Lycaena* species – Friedrich 1986) were observed in the field and in the laboratory, respectively. We observed very few cases of caterpillar predation by spiders, but we definitely also need data on predation on the three stages to get a complete picture of what is a habitat for this species.

Adult mobility and dispersal

Lycaena helle populations have a relatively narrow ecological niche, with well-known needs for at least three out of the four stages. In the Pisserotte population, all the resources needed are spread in the site, flying adults making the link between discontinuous distributions of these resources. This was not the case in Mongolia, where resources were aggregated, leading to shorter local movements (Chuluunbaatar et al. 2009). In our study case, males were flying more often than females, but it did not result in higher emigration rates or dispersal distances (i.e., the same movement kernel for both sexes). Emigration was negatively affected by density, but increased when the proportion of females increased. This is probably associated with the territorial behaviour of males; when there are more females there will be more interactions, and males may even chase unreceptive females out of the habitat patch. This will, in turn, result in more emigration.

Lycaena helle adults are considered to have a low mobility in general, as it has already been shown in other studies (maximum distance of movement observed: 560 m in Fischer et al. 1999; Bauerfeind et al. 2009; 386 m for females and 163 m for males in Chuluunbaatar et al. 2009). The maximum distance of movement recorded was 1132 m within continuous habitat for *Lycaena hermes* (but no between site movements; Marschalek and Klein 2010); 214 m for *Lycaena arota* in sites with scattered host plants (Scott 1973) and 706 m for *Lycaena hippothoe* (Turlure et al. 2010b). Thus, territorial Lycaenidae species are often considered as extremely sedentary based on MRR data.

Nevertheless, Bachelard and Descimon (1999) observed long-distance colonisations in the Morvan region (France). Genetic analysis using individuals from the Ardenne (Belgium) and Eifel (Germany) regions revealed relatively high genetic differentiation between these regions with only limited exchange rates (Finger et al. 2009). We recently

observed the colonisation of empty habitat patches (distant by more than 1000 m from occupied patches) in the Bellemeuse valley (Belgium; Turlure, unpublished data). As stated by Schneider et al (2003), “the classification of a species as sedentary may be related more to the size of the study area than to the species’ actual dispersal ability”. In this case then, distances between recaptures may be viewed as a surrogate for resource grain rather than real dispersal ability.

CONCLUSIONS

In this contribution, we developed an organism-centered, resource-based approach to recognize functional habitat for the focal species of this book, *L. helle*. Recently, this habitat approach has been adopted for several butterfly species (Vanreusel and Van Dyck 2007; Turlure et al. 2009) and is of particular significance for conservation issues (Vanreusel et al. 2007; Merckx and Berwaerts 2010). For butterflies, it is of key importance to integrate the requirements in terms of consumables and specific conditions of both the pre-imaginal and the adult stages. The fact that habitat has often been perceived in a simplistic binary way as discrete and homogeneous patches in a hostile landscape matrix may limit conservation and restoration opportunities (Dennis et al. 2006). This was illustrated here for *L. helle*. The practical use of the resource-based habitat approach has taken advantage from the developments for Geographic Information Systems. However, the risk of this approach lies in the amount and realism of biological information that underlies the maps, especially when maps need to cover relatively large areas, corresponding to the scale at which many practitioners have to take conservation decisions.

This study confirms that this butterfly is linked to transitional and ephemeral biotopes and raises the question of their management and restoration to conserve *L. helle*. Indeed, if tree edges and copses (one of the resources used by adults) have to be preserved or even developed, in the same time, complete (natural) afforestation of the wet meadows or peatbogs should be prevented in order to conserve the set of other resources needed by the species. Therefore, there is a need to find an appropriate equilibrium between intervention and abandonment, using rotational mowing, grazing or tree cutting (Fischer et al. 1999; Goffart et al. 2010; see Chapter XI of this book, Goffart et al.). This must be implemented at the landscape scale too, with the (re)creation of new habitats towards valley heads allowing progressive migration of populations to higher altitudes, with hence a better chance of survival in the context of the current climate warming (Habel et al. 2011).

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Authors' contribution

CT, HVD, PG and NS designed the study. CT collected the data. CT and NS performed the data analyses; all authors commented, interpreted and participated to the improvement of these analyses. CT and HVD wrote the first draft of the manuscript; all authors contributed substantially to revisions.

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Population structure assessment in situ – Mark-Release-Recapture analyses on butterflies

Jessica Weyer

Department of Biogeography, Trier University, D-54286 Trier, Germany

*Corresponding author: Jessica Weyer, Department of Biogeography, Trier University,
Universitätsring 15, D-54286 Trier, E-Mail: jessica_weyer2000@yahoo.de

Mark-release-recapture (MRR) studies are a well established method for the assessment of population densities and mobility for a wide range of species (Hill et al. 1996; Schneider 2003). Particularly since the use of GPS devices became frequently applied in MRR studies, this method offers diverse options to analyse a magnitude of ecological questions concerning populations. By now, it is a common method to analyse population structures, especially for insects.

In the best case, a MRR study starts with the onset of the activity period of the respective study species. During this time, as many people as necessary (depending on the size, or the number of the study area(s) and the number of individuals of the study species) should mark all available individuals of the study species within the study area. It is best to catch and mark the individuals on each day with suitable weather conditions, being important for obtaining a high recapture ratio. In fact, high recapture ratios are the prerequisite for reliable population size estimations. This point will be addressed in more detail below.

For the work with butterflies, each netted individual is marked with an individual code on the underside of the hind wings using a waterproof pen. The code normally consists of one letter (A-Z) for the capture day and a running number. Additionally to the individual code, the following information should be recorded for each capture event: sex, weather conditions, GPS data (e.g. Garmin eTrex Vista HCx) of the capture point (measurement accuracy for the mentioned GPS: e.g. $\leq 3\text{m}$ under suitable conditions), vegetation type at capture point, time of capture, wing wear (1-4 scale: with 1 being fresh and 4 being heavily damaged, cf. Munguira et al. 1997; Zimmermann et al. 2005) and behaviour prior to capture (i.e. flying, mating, feeding, resting and nectaring, nectar plants). These collected data give information about differences in behaviour between sexes, nectar plant preferences, the influence of weather conditions on species activities and the age structure of the studied population.

On the basis of a constantly high recapture ratio (reliable results can be obtained from recapture rates higher than 25%), the total population (N) and the daily population

(N_t) size can be estimated, according to the Jolly Seber method for open populations. An accepted procedure to estimate population sizes (separately for both sexes) is the use of the module POPAN 5.0 integrated in the program MARK v 4.3. (Cooch and White 2007). POPAN estimates three primary parameters: daily survival probability (Φ_t , including mortality and emigration), daily capture probability (p_t) and proportional recruitment (ϕ_{ent_t}) (cf. Fric et al. 2009). Further, the daily population size can be implemented to obtain population trends.

The detailed GPS data of capture/recapture events obtained in the MRR study can be used to analyse the mobility of the respective study species. Calculations of distances can be performed with ArcView GIS 3.2 (ESRI 1996), with the extensions “Animal Movement” and “Home Range Analysis”, or in ArcGIS® 9.3 (ESRI). For those individuals recaptured at least once, it is possible to measure the distance from the day of capture to the day of recapture. Based on these data, “distance per day” and the “cumulative route distance” of each individual through addition all single distances can be calculated. Maximum distances of the individuals recaptured at least twice can be measured between the most distant capture events.

To predict a proportion of individuals moving definite distances, the inverse cumulative proportion of individuals can be assigned into distance classes. Normally, as basis for this analysis, the single distance is chosen (with each distance class representing an e.g. 20 m interval) (cf. Junker et al. 2010). These data are fitted against two mathematical models: the negative exponential function (NEF) and the inverse power function (IPF) (Fric and Konvicka 2007). To exclude artefacts based on the selected interval size, it is advisable to perform the same analysis e.g. with 30 m and 50 m intervals, separately for both sexes. However, the size of the intervals has to be adapted to the results of the respective study.

To exclude the possibility that the observed distances are randomly distributed, the command “Generate Random Points” in the extension “Animal Movement” can be used. Based on these random points, an equal number of random distances can be calculated to compare those with the distances measured.

For individuals captured four or more times (on different days) the size of the used areas can be calculated in ArcView GIS 3.2 or ArcGIS® 9.3. These data provide information about the habitat use (size of the used habitat and type of the area frequently used by the individuals). The two most common methods for this type of analysis are: MCP “Minimum Convex Polygons” analyses and the calculation of 95% kernels.

Furthermore, mark release recapture studies contributed knowledge about the structure of metapopulations and dispersal of species (Baguette et al. 2011) as a number of studies deals with inter-patch movements, an important aspect in metapopulation theory (Hanski and Ovaskainen 2003). While MRR data provides information on dispersal (actual movement ways), a relatively new application is their use for the parameterisation of resistance surfaces in landscape genetics (O’Brien et al. 2006, Epps et al. 2007, Spear et al. 2010).

Mark release recapture studies therefore are an excellent method to enhance our knowledge about population structures and to advance our understanding of important

ecological questions. Nevertheless, some few drawbacks have also to be considered. First, for those individuals never recaptured, no knowledge exists on their fate. Possibly, it is just these individuals that leave the population and some of them travel the largest distances. Second, there is no knowledge about the movement behaviour between two capture events so that only linear distances can be assessed. Therefore, the measured distances and used habitat sizes in MRR studies should be seen as the minimum dispersal potential of a species and the minimum area used by the species.

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Mobility, behaviour and phenology of the Violet Copper *Lycaena helle* in North-Western Romania

Cristina Craioveanu*, Cristian Sitar & László Rákosy

Department of Taxonomy and Ecology, Babes-Bolyai University, RO-3400 Cluj-Napoca, Romania

*Corresponding author: Cristina Craioveanu, Department of Taxonomy and Ecology, Babes-Bolyai University, Str. Clinicilor 5-7, RO-400006 Cluj-Napoca, Romania, E-Mail: cristinacraioveanu@gmail.com

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Abstract

In most Central and Western European countries, the protected butterfly *Lycaena helle* is present in isolated univoltine populations restricted to mountain areas. Populations from Romania differ from most other European ones as they occur in lower altitude and create two generations (bivoltinism). As the Romanian populations occur in that low altitude, they are assumed to go extinct in the near future due to climate warming. During a long-term study, we analysed the distribution, ecology and population structure of *L. helle*. We found a unique phenology of the population of Lapusel (Maramures County) occurring in two generations. This population consists of a high number of individuals and is located at a low elevation, i.e. 170 m a.s.l. The flight distances between consecutive captures are larger than those reported in previous studies elsewhere. However, the overall dispersal behaviour is restricted. This sedentary behaviour in combination with ongoing habitat fragmentation in the wake of habitat destruction (through land-use intensification) might aggravate the current persistence of this highly endangered butterfly species.

INTRODUCTION

The Violet Copper *Lycaena helle* (Denis and Schiffermüller 1775) is a boreo-montane butterfly of the Palearctic and was widely distributed over Central and Eastern Europe during the late glacial and early postglacial period (Habel et al. 2010). Postglacial warming in combination with land-use changes (e.g. afforestation, peat extraction, drainage, transformations into arable land, urbanisation, burning and chemical treatment) caused a severe decline and a recent retraction of the Violet Copper over Central Europe to several small and isolated population remnants, mostly restricted to higher elevations (Kudrna

1986; Fischer et al. 1999; Van Swaay et al. 2010; Kudrna et al. 2011; Van Swaay et al. 2012). The species is considered as endangered in Europe (Van Swaay et al. 2010) and vulnerable in Romania (Rákossy 2002). The habitats of *L. belle* are cool damp grasslands, forest clearings with sheltered warm pockets along with shrubs and trees that are used as perching and sleeping places (Biewald and Nunner 2006; Turlure et al. 2009). The presence of the larval food plant *Polygonum bistorta* is crucial for this species (Habel et al. 2010). The Central European populations are univoltine with one generation from May to July, whereas the Eastern European ones (including Romanian) are bivoltine flying from May to June and July to August (Tshikolovets 2003; Tolman and Lewington 2008). Earlier literature reports bivoltinism also in populations located in the Ardennes (High Vens, Belgium) and parts of Eastern Germany and Poland (Püngeler 1937; Bergmann 1952).

The Romanian populations are at the south-eastern margin of the species' European distribution (Kudrna et al. 2011). Probably, the most serious threat to the lowland populations of this species is climate warming (Settele et al. 2008). The overall European trend for cold adapted taxa is an altitudinal or latitudinal range shift (Parmesan et al. 1999; Varga and Schmitt 2008; Habel et al. 2010). In contrast to this trend, one of the two largest populations of Romania thrives at low altitude in a natural light oak forest, whereas several other previously large populations have gone extinct over the last 100 years as a result of man-made habitat alterations and abandonment of management practices. An assessment of the distribution and conservation status of Romania's *L. belle* populations is needed to complete the information on this species at the European level and to maintain viable populations on the long term. Previous extinctions of large populations in Romania showed a rather high vulnerability of this species towards land-use (change and abandonment of grassland and forest management). Moreover, it is important to determine what management actions are required to reduce the loss of populations in Romania and across Europe.

In our study, we reviewed the existing literature on occurrences of *L. belle* and compared these data with the current occurrences over Romania. We present a comprehensive and complete occurrence map for *L. belle* in Romania. A Mark-Release-Recapture study was performed to assess population size, average lifespan of the adults, flight distances and the preferred activities of the butterflies of this lowland population. Based on these data, we analysed the phenological and ecological characteristics and population structures of a Romanian lowland population. Finally, electron microscopy was conducted to analyse pre-adult stages. We discuss our findings against the background of species conservation.

MATERIAL AND METHODS

Distribution and habitat structure of *Lycena belle* in Romania

All known literature records from Romania were analysed. According to all previously given occurrences and thus habitats, we assessed the present occurrence of the butterfly

in these regions in potentially suitable habitats. For each habitat where *L. helle* still could be found, we assessed the following habitat structures: open area proportion (i.e. grassland/forested area ratio), glades inside forests, and 5 vegetation layers: ground vegetation (short, high), cover of shrubs with a height up to 4 m, proportion of young trees (4-7 m), and proportion of older trees (higher than 7m) (Fig. 1).

MRR study

A viable population of *L. helle*, which exists at low altitude, is situated in Lapusel (Maramures County) (<170m a.s.l.), inside an oak forest with dense ground vegetation and high abundance of *Polygonum bistorta*. The population from this location was used for further analyses. The total area of the forest body in which *L. helle* occurs is 200 ha (Fig. 2). This population consists of several colonies on small suitable habitat patches within this oak forest. The patches are separated by dense vegetation by distances ranging from some few to several hundreds of metres.

We conducted a Mark-Release-Recapture study in a forest area of approximately 64 ha in Lapusel (Maramures County, north-western Romania) during 2011 and 2012. The data from 2011 were exclusively used for estimating flight period, maximal lifespan and flight distances. Mark-Release-Recapture was performed in 2012 during the peak flight periods of the two generations (5-11 May and 6-11 July respectively) to estimate (i) current population size, (ii) average lifespan, (iii) preferred activity, and



Figure 1. Typical forested habitat of *Lycaena helle* in Romania (Photo by C Craioveanu).



Figure 2: Oak forest near Lapusel (Maramures County) with the only known large lowland population of *Lycaena helle* in Romania.

(iv) flight distances of adults. Forest clearings were observed by six persons daily (with one exception due to bad weather conditions) and butterflies were marked between 10 a.m. and 5 p.m. Six and five field campaigns were conducted for the first and the second generation, respectively. The sequence of visits to the clearings was randomised to avoid systematic effects of the time of the day. For each capture event, the following parameters were registered for each marked individual: sex, age (fresh, medium, old), activity (flight, basking, feeding, oviposition, territorial behaviour, mating). GPS coordinates and time were taken for each capture.

Statistics

Data were analyzed separately for each generation with the Cormack-Jolly-Seber type constrained models (Schwarz and Arnason 1996; Schwarz and Seber 1999) using the program MARK 6.0 package (Cooch and White 2010). The fit of the Cormack-Jolly-Seber type models was assessed separately for each generation with the Akaike Information Criterion corrected for small sample size (AICc) (Akaike 1973; Hurvich and Tsai 1989). After running predefined models in Mark, we selected the model with the lowest ΔAICc and the smallest number of parameters, as recommended by Burnham and Anderson (2002). The models considered to best approximate the effects of factors

supported by empirical data are the ones with AICc differing from the minimal one by less than 2. For the whole flight period, the population was estimated with the formula: $N_{\text{total}} = N_{\text{peak}} / (1.9 \hat{e} / \text{FPL} + 0.047)$, where N_{total} is the total estimated population, N_{peak} is the highest estimated population per capture period (peak flight activity day), FPL is the flight period length in days and \hat{e} is the individual lifespan computed using the formula $\hat{e} = (1 - \varphi)^{-1} - 0.5$ (φ is the survival probability) (Nowicki et al. 2005).

The respective parameters resulted from analysing the data with the program Mark 6.0. Survival and capture probability were used to estimate the daily number of individuals for each capture occasion (i.e. days of capture) and the recruitment of new individuals into the population. To compare the daily population estimates between generations and between sexes, we used Kolmogorov-Smirnov Comparison (computed with the program Past, version 2.01 (Hammer et al. 2001)) as datasets were not following a normal distribution.

We calculated the mean flight distance between captures and recaptures and estimated the daily mean flight distance by dividing the mean distance between the points of individual capture and recapture (separately for males and females) by the number of days elapsed between capture events. To compare mean daily flight distances of males with that of females, we used the Kolmogorov-Smirnov test (computed with the program PAST, version 2.01 (Hammer et al. 2001)) because the datasets deviated from normal distributions.

RESULTS

Distribution and habitat structure of *Lycaena helle*

The current information on *L. helle* in Romania is based on rather old data from field observations and faunistic studies and therefore lacks quantitative data (Salay 1910; Czekelius 1899; Rebel 1927; Ciochia and Brătășeanu 1979; Izsák 1982; Szabó 1982; Rákossy and Weber 1986; Burnaz 1993). Today, the Violet Copper exists in two large populations in Romania: one situated at an altitude of about 500 m a.s.l. (Vad, Brasov County) and another at about 170 m a.s.l. (Lapusel, Satulung Commune, Maramures County). Besides these two still viable populations, five populations have to be considered as extinct: Chitila (Salay 1910), Vlădeni (Ciochia and Brătășeanu 1979; Izsák 1982), Sighisoara (Czekelius 1899; Rákossy and Weber 1986), Livada 1 (Szabó 1982), Cernei Gorge (Rebel 1927; Rákossy and Neumann 1997). Furthermore, several other small populations are assumed improbable to survive over the next ten years (Szabó 1982; Burnaz 2002, personal comm. Z. Laszlo) (Fig. 3).

The Romanian populations of *L. helle* prefer damp forested habitats with a mosaic structure alternating between open spaces and more densely forested areas. Typical habitats are structured in five vegetation layers: a ground layer with high abundance of *P. bistorta*, a tall perennial level dominated by *Filipendula ulmaria*, a shrub level with



Figure 3: Distribution of *Lycaena helle* populations in Romania (red x = extinct, green = confirmed populations after the year 2000).

Ligustrum vulgare, a level of young trees of low height (4–7 m) and a layer with old-growth trees (Fig. 1.). The areas with larger numbers of individuals offer the necessary nectar sources to the adults and are sunny and wind protected throughout the daily activity period (10 a.m. – 5 p.m.). The first *L. helle* generation of the lowland population in Lapusel (Maramures County) starts at the end of April and ends at the end of May. The second generation starts at the end of June and ends at the end of July.

Dispersal behaviour and population structure

At the peak of the first generation flight period, 5–11 May, we marked 1,853 individuals (1,039 males and 814 females) of which 326 individuals (18%) were recaptured on different days. The recapture rate in females (17%) was slightly lower than for males (18%). At the peak of the second generation flight period, 6–11 July, we marked 482 individuals (294 males and 184 females) with a total recapture rate of 21% (101 individuals). The recapture rate in females (16%) was lower than of males (24%). In 2011 when the whole flight periods were sampled, both males and females were recaptured after a maximum of 25 days in the first generation and after 11 days in the second generation.

For the first generation, a population of 9,650 individuals ($\pm 2,645$ SE) was estimated with a mean individual lifespan of 7.6 days (± 2.0 SE). The model $\Phi(\cdot)p(t)$ (i.e. the model with constant survival probability and different capture probability over time) was selected for the estimations of the first generation. Survival rate was estimated at

0.88 (± 0.03 SE) and capture probability at 0.09 (± 0.01 SE). For the second generation, a population of 4,741 individuals ($\pm 1,120$ SE) was estimated with an individual lifespan of 3.3 days (± 0.8 SE). The model $\text{Phi}(g)p(t)$ (i.e. the model with different survival probability for females and males and different capture probability over time) was selected for the estimations of the second generation. Survival rates were estimated at 0.76 (± 0.1 SE) for females and 0.73 (± 0.2 SE) for males and capture probability at 0.16 (± 0.04 SE).

Considering the population estimates per capture occasion (N_i), the first generation was more numerous than the second (Kolmogorov-Smirnov test: $D = 1, p = 0.002$). More males than females were estimated, both in the first and in the second generation (Kolmogorov-Smirnov test: $D = 0.83, p = 0.01$ and $D = 0.80, p = 0.03$, respectively) (Fig. 4).

The mean distances between consecutive recaptures were 111 m for females and 135 m for males, whereas the mean daily flight distance for females was 39 m and for males 56 m. Mean daily flight distances and distances between consecutive recaptures did not differ significantly between males and females (Kolmogorov-Smirnov tests, $p > 0.3$). 60% of the recaptured individuals flew less than 120 m, further 24% 120 to 210 m, and 14% 210 to 540 m. Only two individuals were recaptured at a distance of up to 590 m.

The majority of individuals of the first generation were captured in flight (53%), 25% while sitting/basking on vegetation (Fig. 5.b-c), 11% while ovipositing, 4, 6% while feeding, 4% while displaying territorial behaviour and 1% while mating (Fig. 5.a). Adults from the first generation mostly feed on *Polygonum bistorta* (Lapusel: 96%), but also on other available nectar sources like *Myosotis* sp., *Cardamine pratensis* (both species not observed in Lapusel, but in other populations from Romanian), *Anemone nemorosa* (Fig. 5.p), and *Ranunculus acris*.

In the second generation, 39% of the individuals were captured flying, 35% while sitting/basking on vegetation, 13% while displaying territorial behaviour 7% while ovipositing, 4% while feeding and 1% while mating. Adults of the second generation have less flower heads of *P. bistorta* available and mostly feed on *Ligustrum vulgare*. Burnaz (2000) also mentions *Mentha longifolia* and *Eupatorium cannabinum*. Both generations also use mineral sources from mud, excrements and, occasionally, human sweat (Fig. 5.q).

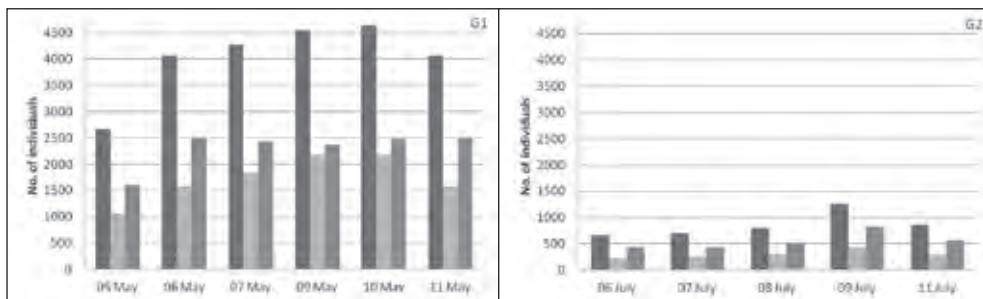


Figure 4: Population estimates per capture occasion for females (light grey), males (medium grey) and the total number of individuals (dark grey) in both generations (G1 and G2 peak flight periods) of *Lycaena helle* in Lapusel (Maramures County, Romania).



Figure 5: *Lycaena helle* in Romania: a-c adults from the first generation; d-e female from the first generation; f-i males from the first generation; j-k female from the second generation; l-o males from the second generation; p adult feeding on *Anemone nemorosa*, q adult feeding on excrements. Pictures provided by: a-c R Wasley, d-o L Rákósy, p-q C Craioveanu

In Lapusel, the feeding individuals were mostly (88%) observed on mud; nectaring individuals were observed on *Ligustrum vulgare* and *Hypericum sp.* More males were captured flying than females (41% and 31%, respectively), 31% of the females were captured while displaying oviposition behaviour and 17% of the males displayed territorial behaviour. 6% of the females and 4% of the males were captured while feeding. 27% of the females and 38% of the males were captured while sitting/basking.

Males show territorial behaviour singly or in small groups guarding areas of few square metres and hunting away other intruding males, but also other butterfly species. The males guard their territories from high observation points located on the top of shrubs. Copulated females display typical oviposition behaviour by flying low over areas with dense *P. bistorta* cover. They search for suitable larval habitat and settle on several bistort leaves before choosing one on which it walks around to find the best position to lay a single egg. The eggs are generally laid singly on the underside of the bistort leaves,

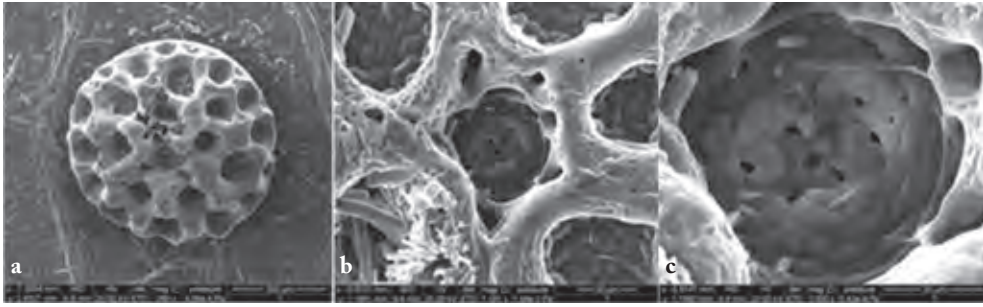


Figure 6: Microstructures of an egg of *Lycaena helle*, obtained from electron microscopy (a) with two different levels of magnification of the micropylar area (b and c) (Photos by C Mihali).

near to the edge of the leaf. The egg is whitish, has a slightly flattened sphere shape and a coarse honeycomb structure, with a diameter of approx. 0.6 mm. The micropylar area is slightly deepened and has a diameter of approximately 80 μm (Fig. 6.).

The larvae exclusively feed on the underside of *P. bistorta* leaves, the first three instars producing a specific feeding pattern (Fig. 7.a). Last instar larvae feed on whole leaf pieces at the tip of the bistort leaf, leaving only the central rib untouched. The pupation process has not been observed in nature. The cremaster of the pupa is covered in hooked bristles (Fig. 7.c-7.d). The period of preimaginal development lasts approximately one month for the second generation. Both generations are distinguished in wing size and wing colouration (Fig. 5.d-o). The males of the second generation are less violet-iridescent and therefore they seem to be darker. The orange colour on females' dorsal side of the wings is very variable within each generation.



Figure 7: Feeding pattern from a larvae of the first instars on the underside of *P. bistorta* leaves (a); Larvae in the third instar (b); scanning electron microscopy image of a pupa with a magnification of the cremaster (c and d); pupae with a silken girdle (e). Photo a by C Craioveanu, b & e by L Rakosy, c-d by L Barbu.

DISCUSSION

Population trends of *Lycaena helle* in Romania

Our literature research and field data showed remarkable differences between old and new data, and many populations have recently become extinct. This negative trend caused the classification of *L. helle* into the vulnerable category (Rákossy 2002), although the given IUCN status of this species in Romania in the Red Data Book of European butterflies (van Sway et al. 2010) is rare.

In contrast to the European trend of this cold adapted species, several populations of *L. helle* in Romania thrive at low altitudes and have an exceptional phenology, without a tendency to retreat to higher altitudes. Several of the historically documented populations were situated under 200 m a.s.l. and the largest still existing population (from Lapusel, Maramures County) is also at an altitude of maximum 170 m a.s.l. Furthermore, the population extinctions in the last 100 years occurred rather due to short-term man-made habitat alterations and abandonment of land-use. Unlike to the areas in Romania from where the butterfly disappeared (Sighisoara region (Rákossy and Weber 1986), Chitila – Bucharest region – (Szabo 1982), Cernei Valley (Rákossy and Neumann 1997)), forest management in Lapusel (i.e. selective cutting) has fostered a good development of the ground vegetation and the persistence of high soil moisture, thus creating optimal conditions for the butterfly population to persist.

Considering only the high soil moisture regime and the presence of oak forests with low inclinations, other populations of *L. helle* had to be assumed to exist across wide areas in Romania, based on GIS modelling (overlay method) (personal comm. A. Crişan). However, this is not the case. Previous studies (Rákossy, unpublished data) showed that another large population of *L. helle* in Romania (Vad, Brasov County), harbouring approximately 5,000-10,000 individuals, is also starting to decline slowly. Here, no drainage has been conducted as this site is a nature reserve. However, the total lack of wood exploitation in this area resulted in a denser canopy over the last few years and, consequently, a less developed ground vegetation cover. Although we did not conduct a mark-recapture study in this area, butterflies were visibly less abundant in Vad in 2011 and 2012 than in the Lapusel site, and most observed butterflies were found in island-like glades.

Another example for this negative population trend is the Livada 1 population (Satu-Mare County). This population was considered very large in the period 1970-1980 (Szabó 1982), but dramatically declined until the period 1995-2000 when observations evidenced a complete extinction (Rákossy unpubl.). In 2010, this population was classified as extinct and individuals of *L. helle* have not been observed in this area until today. In this specific case, no other factor than natural succession of the forest that lead to the disappearance of the glades and reduction of the host plant can be incriminated. The Livada 2 population was reported from very recent observations (László Zoltán) in a closeby (3 km) forest body with more diverse habitat structures than in Livada 1.

Ecology of *Lycaena belle* in Romania

The studied lowland population of *L. belle* from Lapusel is a large bivoltine population, with an estimated number of individuals of more than 14,000 individuals (both generations). Recapture rates were lower for females than for males in both generations. Equal survival rates for males and females and different capture probability over time (as found in the first generation), due to changing weather conditions, are typical for adult butterflies (Nowicki et al. 2009). However, the model for the second generation revealed different survival probabilities for males and females, maybe representing an artefact due to the large differences in the number of marked and recaptured females and males. The behavioural differences between sexes might explain the lower catchability of females, especially in the second generation. This is indicated in our study by the higher rate of males captured during flight than females. Previous studies on *L. belle* also showed that males are more active and therefore more visible than females; furthermore, they are also more stationary, controlling their territory (Fischer et al. 1999). However, the male/female basking ratio is different in our study. The high proportion of males sitting or basking might be due to the inclusion of males perching in high observation sites while defending their territory into this category of behaviour.

The mobility of *L. belle* in the investigated Romanian lowland population is relatively high if compared to previous studies elsewhere. Although between populations movement might be practically impossible due to their restriction to isolated natural oak forest bodies, the mean flight distances between recaptures proved to be larger in Lapusel than previously documented from studies of *L. belle* in open-land habitats (Fischer et al. 1999; Chuluunbaatar 2009). The lower mobility in German populations from Rhineland-Palatinate could be explained by the higher isolation of suitable grassland patches within an old-growth spruce afforestation (Fischer et al. 1999).

While the suitable habitat for *L. belle* in Lapusel stretches over large areas inside the natural oak forest. Suitable habitat patches within the forest are interconnected because of different ages of trees and the presence of five layers of vegetation. Shrubs and higher vegetation structures (forest edges) have been previously shown to offer wind-sheltered stands and represent dispersal pathways (Fischer et al. 1999). Thus, the natural oak forest of Lapusel of mixed tree ages, managed by selective cutting, does not hinder the movement of butterflies across unsuitable habitat (e.g. old-growth forest with no ground vegetation/low insolation). Considering the flight distances, unsuitable habitat area would have to extend 120 to 600 m in all directions from a glade to represent a real barrier for *L. belle*.

While the Mongolian populations from West Khentey region occur in an open habitat complex, a mosaic of habitats alternating with mesophilic herb meadows with shrub layers and bog areas, and showed larger flight distances between recaptures than the German populations (Chuluunbaatar 2009), their mobility is still lower than that of the Romanian Lapusel population.

The open-land habitats surrounding the oak forests in the region of Lapusel seem to represent real barriers, and no individuals of *L. belle* were found here. These findings are

supported by Fischer et al. (1999) stating that open fields act as barriers for dispersal due to lack of wind-sheltered stands. In a nearby and mostly similar forest body, we have found another population of *L. helle*, but individual exchange between both is unlikely as they are separated by a minimum of 6 km of grasslands, fields and urban areas. A connection between suitable forest habitats could be re-established if forest strands would be kept along river sides.

Conservation of *Lycaena helle* in Romania

The close connection of Romania's *L. helle* populations with forested habitat makes this species especially vulnerable to forest management. The forest populations of *L. helle* are affected by the availability of the larval food plant (high soil moisture) and by the presence of multiple layers of habitat structures (Van Swaay et al. 2012). Moreover, because of the low flight distances, the connectivity of suitable habitat (forest glades with *P. bistorta* and typical vegetation layers) is important for individual exchanges within a metapopulation. Thus, natural succession plays a critical role in preserving the populations of this butterfly in Romania: Lack of appropriate management of forested habitat will lead to a decline in the populations of this species. Abandonment will lead to canopy closure with progressing age of the trees (10-100 years). On the other hand, a forest management allowing clear cuts of the whole forest body or large area cuts at the edge of forest body will reduce typical habitats for a larger number of typical forest butterflies, leading to their decline (Crisan et al. 2012). The forest management strategy proven to be beneficial for entire forest butterfly communities and also for the specific populations of *L. helle* in Romania is selective cutting inside the forest body increasing the overall forest edge and creating small glades (Crisan et al. 2012). The good status of the Lapusel population is due to the combination of selective cutting inside the forest body with periodical planting of tree saplings, ensuring the persistence of different tree ages. This management preserves a number of forest glades with all layers of structures beneficial to *L. helle* and their good interconnectivity.

Considering the large (52%) reduction in forested area of Romania in the last century (Biris and Veen 2005), typical forest butterfly communities have suffered considerably (Schmitt and Rákósy 2007). In this context, the still existing populations of *L. helle* probably represents a small proportion of the ones that might have existed hundred years ago in the north-western part of Romania. Due to drainages and change in the destination of areas to arable land, other fragile populations of different butterfly species disappeared from Transylvania already hundred years ago (Czekelius, 1899, Rákósy and Weber 1986). As Romanian populations of *L. helle* are at the edge of the species' European distribution and very isolated, they might represent relict populations with an independent evolutionary history. Similar studies on *E. maturna* have shown that populations isolated at distribution edges represent a significant evolutionary unit (Rákósy et al. 2012). Thus Romania has a high responsibility in preserving these last relict populations of this butterfly species.

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Biogeography: From the analysis of distributions to the distribution of genetic variants

Thomas Schmitt^{1,2*} & Jan Christian Habel³

¹Department of Biogeography, Trier University, D-54286 Trier, Germany

²Senckenberg German Entomological Institute, D-15374 Müncheberg, Germany

³Terrestrial Ecology Research Group, Department of Ecology and Ecosystem Management, Technische Universität München, D-85350 Freising-Weihenstephan, Germany

*Corresponding author: Thomas Schmitt, Senckenberg Deutsches Entomologisches Institut, Eberswalder Straße 90, D-15374 Müncheberg, Germany, E-Mail: thomas.schmitt@senckenberg.de

Biogeography is a scientific discipline, which is fascinating scientists since long. One of the main items of this discipline is the understanding of the past shifts of distributions of animal and plant species and thus to understand their range dynamics. One important question of this discipline is the detection of the last dispersal centre(s) of a species. During the past 25 years, the techniques available for biogeographic analyses have largely increased and thus have led to strong dynamics in this scientific field.

Until the early 1980s, most biogeographic analyses followed the classical methods of the interpretation of distribution patterns, i.e. chorological analyses as e.g. performed by Reinig (1937, 1938, 1950), de Lattin (1949, 1964, 1967), Holdhaus (1954) and many others. In these approaches, the distributions of many species were superimposed and the areas with high overlap of species distributions were considered as the core areas, which should include the last dispersal centres for a larger group of species. These dispersal centres were further tested by analyses of the subspecific structures. If such a core area of species distribution at the same time was a centre of subspecific differentiation, this combination strongly enforced the interpretation. Based on such analyses, the old biogeographers distinguished different faunal elements (i.e. taxa having the same centre of dispersal) and faunal regions (i.e. regions with a characteristic species assemblage). Note that all faunal elements always include a refugial area (i.e. a dispersal centre), whereas faunal regions do not. Faunal regions can have a very specific species composition for other reasons than being a refugium (cf. Dennis et al. 1991; Heiser & Schmitt 2010).

Advances in statistics have largely enhanced this field of classical biogeography. In contrast to the old often intuitive analyses of faunal elements and regions, new statistic methods, as e.g. used by Dennis et al. (1991, 1995, 1998), Heiser & Schmitt (2010) and Krefts & Jetz (2010), allowed to calculate the distribution of faunal elements and regions

from raw data sets without any *a priori* assumptions and to generate the borders between different realms by using mathematical algorithms.

More recently, the modelling of past, recent and even future distribution patterns of species under different climate change scenarios has largely enhanced our understanding of biogeography and the debates on range fluctuation among scientists (as also projected for *L. helle*, Habel et al. 2010, 2011) (see chapter IX of this book). However, the resulting “distribution maps” have to be analysed with caution as they simply reflect probabilities under the climatic niches given by the presence points provided by the user of one of these respective programs. Therefore, it is highly recommendable to back-up the interpretation of such models by using some other analytical tools in biogeography research, as genetic data.

The application of molecular tools and the analyses of genetic structures over larger geographical ranges is becoming more and more popular and displays an independent research field, named phylogeography by Avise et al. (1987). In principle, these phylogeographic analyses are doing the same as the old biogeographical analyses of distribution patterns, but not subspecies or morphological features are mapped on geography, but different alleles of genes or gene fragments. At the beginning of these molecular analyses, allozymes were a frequently used marker system, which subsequently was substituted more and more by the sequencing of mitochondrial or chloroplast genes; actually often being completed by additional sequencing of chromosomal genes (see chapter IV of this book). As in the classical analyses, advances in the available statistics have largely enhanced the analytical capacity of phylogeographic analyses. Although debates on the reliability of some of these analyses are quite controversial and ongoing, these statistical advances have considerably fuelled biogeographical debates.

The combination of all these techniques have strongly enhanced our understanding of biogeography all over the world, but especially in Europe and North America (e.g. Hewitt 1996, 1999, 2000, 2001, 2004a, 2004b; Comes & Kadereit 1998; Taberlet et al. 1998; Schmitt 2007, 2009; Schmitt & Varga 2012). However, all these modern analyses have also supported many of the old assumptions hereby underlining the high scientific level of the old biogeographers working with quite simple methods, but with brilliant brains.

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The molecular biogeography of the Violet Copper *Lycaena helle*

Jan Christian Habel^{1*}, Marc Meyer², Thomas Schmitt^{3,4,5}, Martin Husemann¹
& Zoltan Varga⁶

¹Terrestrial Ecology Research Group, Department of Ecology and Ecosystem Management,
Technische Universität München, D-85350 Freising-Weihenstephan, Germany

²Orscholzer Straße 15, D-66706 Perl-Kesslingen, Germany

³Department of Biogeography, Trier University, D-54286 Trier, Germany

⁴Senckenberg German Entomological Institute, D-15374 Müncheberg, Germany

⁵Institute of Biology/Zoology, University of Halle, D-06120 Halle (Saale), Germany

⁶Department of Evolutionary Zoology, Faculty of Science and Technology, University of
Debrecen, H-4010 Debrecen, Hungary

*Corresponding author: Jan Christian Habel, Terrestrial Ecology Research Group,
Department of Ecology and Ecosystem Management, Technische Universität München,
Hans-Carl-von-Carlowitz-Platz 2, D-85350 Freising-Weihenstephan, E-Mail:
janchristianhabel@gmx.de

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Abstract

The upper Pleistocene is characterised by strong climatic oscillations. Long glacial periods were interrupted by relatively short and warm interglacial stages. These climatic changes have periodically led to severe range shifts of biota. During the cold phases, the European lowlands north of the high mountain systems were covered by tundra-steppe habitats. Thus, suitable habitat for the Violet Copper butterfly *Lycaena helle* was possibly available over major parts of Central Europe. These areas might have fulfilled all habitat requirements of the species, like the presence of one of its food plants, i.e. *Polygonum viviparum*, but retreated in the wake of postglacial warming to small remnants. The genetic structure of the species studied using polymorphic microsatellites, allozymes and mitochondrial DNA sequences suggest a wide distribution during the cold phases of the past and relatively fast altitudinal and latitudinal range shifts during the postglacial warming, accompanied by strong population fragmentation of the remaining extant populations until today. As *L. helle* is not found in Denmark, the postglacial colonisation of Fennoscandia most probably has taken place via Finland to Sweden and Norway. This colonisation direction is in concordance with the detected genetic structures: a successive elimination of genetic information in the migration direction. All over the Central European lowlands, the suitable climatic niche

has been shrinking to small retreats limited to the archipelago-like higher elevations of the European Middle Mountains. On these mountain islands, *L. belle* persists in small and isolated relict populations until today. The isolation of these mountain populations has led to remarkable genetic differentiation accompanied with genetic uniqueness evolving in each mountain area. Mitochondrial haplotype diversity is extremely low, suggesting recent bottlenecks, relatively low population sizes and probably a fairly young isolation history and therefore the lack of lineage sorting.

CLIMATE AND ECOSYSTEMS IN CENTRAL EUROPE DURING THE COLD PHASES OF THE PLEISTOCENE

Recent climate change affects the physiology, phenology and the distribution of plant and animal species all over the world. Plants and animals are responding in an overall unsurprising way; they react as they did at the end of the last ice age, the Würm glacial. Strong range shifts of European species can mainly be seen as the species' efforts to maintain their relative distribution within that part of the temperature zone they are adapted to (Hewitt 2004). Therefore, to better understand the recent and future range changes of species, it will be crucial to understand their distribution history and, in particular, their reactions to the glacial-interglacial oscillations of the Quaternary.

In this context, proxy indicators such as coral reef records, tree rings, marine sediment cores and ice core records evidence these previously described strong climatic changes over the past 2.5 Myr (Quante 2010). This '*ice age era*' represents the most recent period in the earth's history of the Kainozoic (Imbrie and Imbrie 1979). The climatic changes were caused by periodic variations in the earth's orbit particularly affecting the summer-insolation at high latitudes (Berger 1988; Lomolino et al. 2006). These glacial-interglacial oscillations of the Pleistocene caused strong changes of the climate resulting in important distribution range changes and adaptations of species. In general, the much longer glacial periods of the late Pleistocene of about 100,000 years were interrupted by much shorter interglacial stages (10,000 to 26,000 years), which were characterised by much higher temperatures (Lomolino et al. 2006).

During the Würm glaciation, the vegetation of Central Europe transformed into cold tundra-steppe habitats. However, recent results of surveys on the late Pleistocene clearly contradict the earlier assumed "*tree-less tundra*"-model (Huntley and Birks 1983; Frenzel 1992; Huntley and Allen 2003; reviewed by Schmitt and Varga 2012) of Europe north of the mountain ranges of the Pyrenees, Alps and Carpathians. Fossil pollen data and macrofossil remains indicate that several tree species have survived at the southern edge of the cold and dry steppe-tundra area in Central and Eastern Europe (e.g. Steward and Lister 2001; Magri et al. 2006; Bhagwat and Willis 2008; Magri 2008; Provan and Bennett 2008). Cold steppe vegetation with scattered boreal coniferous forest fragments have been shown to have occurred during the cold stages e.g. in southern Moravia and in several parts of the Carpathian Basin (Willis and Andel 2004; Bhagwat and Willis 2008; Birks and Willis 2010; Varga 2010). This scenario has also been underpinned by studies on the late Pleistocene mammalian fauna as the carrying capacity must have been

sufficient to support numerous large herbivores such as mammoth, woolly rhinoceros, reindeers, giant deer, bison or aurochs' demanding a highly productive environment (Guthrie 1990; Stewart and Lister 2001; Simakova 2006; Danukalova et al. 2009).

These former ecosystems of the Central European lowlands during the cold stages might have been comparable with the extant cold-continental meadow steppes in southern Siberia or northern Mongolia (Varga et al. 1989, 2010). These mammal assemblages also might have included cold-tolerant small mammal species of temperate habitats (e.g. *Sorex araneus*, *Clethrionomys glareolus* and *Apodemus sylvaticus*, Wójcik et al. 2002; Kotlík et al. 2006). Furthermore, cold-tolerant frogs and reptiles such as *Rana arvalis*, *Zootoca vivipara* and *Vipera berus* survived in extra-Mediterranean core areas north of the high mountain systems of Europe (Surget-Groba et al. 2001; Babik et al. 2004; Ursenbacher et al. 2006; Joger et al. 2007; Saarma et al. 2007; Stewart et al. 2010).

Based on our paleo-ecological knowledge, a wide distribution of the butterfly *L. helle* in these periglacial tundra-steppes during the late glacial and early postglacial period is most likely. Such a distribution pattern is further supported by paleo-botanical data and pollen records confirming high abundances of the species' larval food plant (*Polygonum viviparum*) in the Central European grasslands about 15,000 years ago (Mägdefrau 1953). Other habitat requirements (like shrubs and trees, nectar sources and a specific succession stage, more details about habitat requirements of larvae and imago are given in Turlure et al. 2009 and chapters I-V, XI, XII; some examples of habitats are given in Figure 1) likely also were available during these late glacial periods in Central Europe. Despite the rather dry climate in Central Europe during the late Würm glaciation, sufficient damp grassland sites should have been available as accumulation of melt water in little depressions in the landscape resulting from snow accumulating during the long winters. *Lycæna helle* currently has a wide continuous range from Belarus and the European part of Russia to southern Siberia and northern Mongolia, inhabiting similar habitat types to the ones that were assumed to be widespread in Central European lowlands during the past glacial phases.

The transition from the last glacial to the current interglacial epoch was dramatic. 12,000 years bp, the Laurentian ice-sheet in North America was releasing large quantities of melt water into newly formed lakes and rivers setting the stage for flood events, which shaped the North American landscape as it is known today with the Great Lakes being the small remnants of these events. Like North America, Europe was similarly modified and most northern European and high mountain glaciers vanished; yet without similar spectacular flooding.

The Holocene is the most recent period of the earth's history and represents the postglacial warming period (Quante 2010). This recent inter- / postglacial climate type is characterised by the expansion of wooded habitats and the regression of non-arboreal environments (steppe, tundra and orol grasslands). Transitional macro-ecotones developed at the forest belt fringes with intermixing of forests with tundra and steppe elements. Today, these ecotones can be found in Fennoscandia and higher elevations of the mountains of Central Europe, in eastern parts of Europe, and southern parts



Figure 1: Habitats of *Lycaena belle* over the western Palearctic. Meadows at the arctic circle in Fennoscandia (Kuusamo, Finland) (a), meadows in the Jura mountains of Switzerland (b), a habitat in the coniferous forests of the Alps (Garmisch-Partenkirchen, Germany) (c), at meadows of the Alps (Mauterndorf, Austria) (d), a habitat in Lithuania (e), a habitat in the Eifel region of western Germany (f), a habitat in the Massif Central of France (g), and collecting individuals of *L. belle* in a habitat close to Sidsjö, Sweden (h).

of Siberia and northern Mongolia. In the latter regions, wet meadow species, such as *Maculinea teleius*, *M. nausithous*, *Lycaena dispar*, *Brenthis ino*, *Proclossiana eunomia*, are constant components of the tall-forb, tall grass meadow steppes.

RETREATS AND EXPANSIONS

Most studies on European biogeography have addressed the genetic structures of thermophilic organisms. These species follow a temperate climatic niche, as revealed for invertebrates (e.g. Schmitt et al. 2003, 2005; Habel et al. 2005, 2009, 2011a, b), vertebrates (e.g. Seddon et al. 2000; Paulo et al. 2001, 2008; Podnar et al. 2005; Carranza et al. 2006; Ursenbacher et al. 2006), and plants (e.g. Comes and Kadereit 1998; Taberlet et al. 1998; Hewitt 2004). In these studies, distinct Pleistocene refugia in the three Mediterranean peninsulas (Iberia, Italy and Balkan) south of the European high mountain systems (Pyrenees, Alps and Carpathians), but also in Northwest Africa and Asia Minor have been detected. Caused by the postglacial warming, these species expanded northwards following various (re)colonisation pathways (classified into four paradigms, cf. Hewitt 1999; Habel et al. 2005). These temperate species have mainly derived from Mediterranean refuge populations and underwent range expansion in the late glacial and the postglacial period (Hewitt 1996; but see Schmitt and Varga 2012 for exceptions).

While these thermophilic organisms went extinct over major parts of Central Europe during the cold phases, many cold adapted species might have reached their maximal expansion over Central Europe during these periods. The Violet Copper *L. belle* is adapted to cool and moist habitat conditions (Habel et al. 2011a). The recent European distributions of many members of this cold-adapted group ('*holopsychric*' type *sensu* Rebel (1931)) have been considered the result of a postglacial "Siberian" invasion for a long time (Hofmann 1873; de Lattin 1964). However, many contradicting evidences of strong intraspecific differentiation between north-eastern boreal and southern European montane populations have been observed, for example for numerous butterfly species (e.g. Varga 1975, 1977; Nève 1996).

Several authors, therefore, have suggested an alternative mode for the postglacial colonisation of Central and Northern Europe by populations expanding from so-called extra-Mediterranean refugia in Central Europe, the Carpathian Basin, the southern Urals and the Caucasus (e.g. Polyakov et al. 2001; Schmitt and Seitz 2001; Stewart and Lister 2001; Surget-Groba et al. 2001; Babik et al. 2004; Ursenbacher et al. 2006; Saarma et al. 2007; Stewart et al. 2010). Other species exhibit mixed patterns with postglacial colonisation from both, Mediterranean and extra-Mediterranean refugia (e.g. Fumagelli et al. 1996; Deffontaine et al. 2005; Kotlík et al. 2006; Magri et al. 2006; Schmitt 2007; Magri 2008).

The glacial periods have been characterised by a regressive fragmentation of wooded habitats and, consequently, by a broad contact of the tundra and the steppe zonobiomes with some forested "pockets" north of the refugial belt of the Mediterranean area. These ice age communities of Central Europe composed by mixing tundra,

steppe and boreal elements regressed during the warmer interglacial and were mostly replaced by elements of the largely expanding forest areas. However, some of these ice age elements remained as relicts at ecologically and climatically specific sites; one such example is *L. helle*.

OUT OF THE COLD STAGE GRASSLANDS

Many cold-adapted species escaped the postglacial warming by altitudinal and/or latitudinal range shifts to cooler climates populating the high mountain systems, the highest parts of the mountain systems with medium elevation and the arctic realm (e.g. Varga and Schmitt 2008). The application of genetic markers during the last few decades has confirmed and modified many earlier biogeographic assumptions on cold-adapted species, but has also raised new questions on glacial distribution patterns and postglacial range shifts with often highly complex reactions to climate modifications (Schmitt 2009). In this context, rather little is known for the group of boreo-montane taxa of which *L. helle* is one representative. These species represent a mix of characteristics of continental, arctic and alpine species. However, rather few genetic analyses are available for this biogeographic group, with examples for some plant species such as *Picea abies* (Lagercrantz and Ryman 1990), *Trollius europaeus* (Despres et al. 2002), *Polygonatum verticillatum* (Kramp et al. 2009), *Cicerbita alpina* (Michl et al. 2010) and the butterfly *Proclossiana eunomia* (Nève 1996).

Lycaena helle has been subject of a variety of molecular studies using allozymes and microsatellites (Finger et al. 2009; Habel et al. 2010, 2011b). Each marker type has its own strength and has provided different types of information on the biogeographic history of the species. A microsatellite study on populations covering the entire western Palearctic distribution range has shown strong genetic differentiation of most populations (Habel et al. 2011a). The neighbour-joining dendrogram suggests that the populations from Fennoscandia represent a monophyletic group (Fig. 2). The genetic differentiation of these populations from the eastern European ones increases from Finland to Sweden. The genetic differentiation among the Fennoscandian samples (whose age cannot be older than the postglacial colonization of this region) is about half of the differentiation of the Finnish populations from the genetically closest other European populations. This supports a recent (i.e. postglacial) separation of the Fennoscandian group from the other European populations and one single colonization route to the North. The relatedness of the Fennoscandian populations with the ones from eastern Europe, the decrease of genetic diversity from Finland to Sweden and the complete lack of this butterfly species in Denmark and southern Sweden (Tolman and Lewington 1997) allows the reconstruction of the colonisation of Northern Europe: most likely, *L. helle* followed the melting ice shield northwards during the early postglacial period (less than 11,600 years ago), and colonized Fennoscandia via the Baltic lowlands, but did not use a western route via Denmark to Sweden.

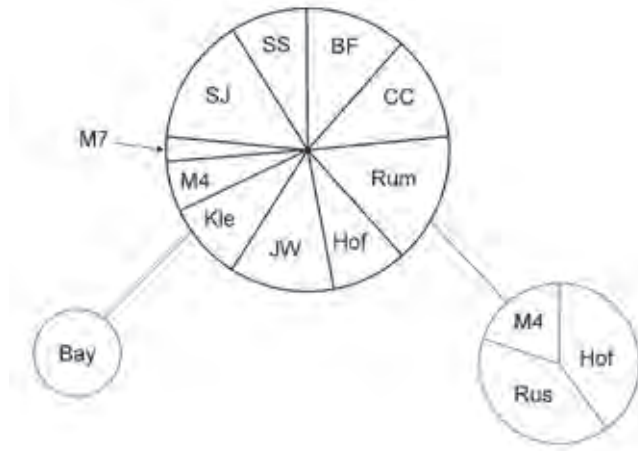


Figure 3: Haplotype network constructed for 41 specimens sequenced for a 629 bp fragment of the mitochondrial *COI* gene. Number of segregating sites, $S = 2$; total number of mutations, $\text{Eta} = 2$; haplotype diversity, $H_d = 0.302$; nucleotide diversity, $\text{Pi} = 0.0005$. Abbreviations: BF, SJ = Fennoscandia; CC, Rum, JW = Eastern Europe; M4, M7 = Massif Central; Hof = Westerwald; Bay = Bavaria, southern Germany; Kle = Eifel; Rus = Russia. Respective genetic sequences were deposited to gene-bank (KC208818- KC208849).

the distribution range (from Western Europe to Russia). The few haplotypes found are shared across large geographic distances indicating extensive bottlenecks and / or fast colonization sweeps.

In order to further study short term dynamics of the species, we tested the temporal changes over a 15 years period for populations from France and Belgium using polymorphic microsatellites. Hereby, we showed that no important changes in allele frequencies occurred over this short time period. These data underline that the genetic structures observed must have evolved at the time scale of thousands of years, but most likely are not older than the Last Glacial Maximum (Habel et al. 2011b).

The assumed pattern of a wide potential distribution of *L. helle* during the LGM and the lack of geography-dependent genetic differentiation of the populations from eastern Europe and the Alps (southern Bavaria, Austria, Switzerland) support the idea of an interconnected network of populations in this region in the past. This assumption is also supported by the results of the analyses of the mitochondrial *COI* gene for 41 specimens across large parts of the species range. No genetic differentiation over the entire western Palearctic distribution was observed and only three haplotypes being scattered over the entire European distribution range were found (Fig. 3). These genetic data support (i) an interconnected distribution over Europe in the past with only recent (i.e. postglacial) fragmentation into distinct mountain populations or (ii) a very fast phalanx-wise expansion from the East, and thus a lack of genetic differentiation (e.g. population subdivision) for the mitochondrial gene analysed. In either case, population bottlenecks and small population sizes have probably led to the genetic pauperization of the species.

At the current western distribution edge of *L. helle*, populations are characterised by strong genetic differentiation obtained from microsatellite and allozyme analyses (Habel et al. 2008, 2011a), coinciding with the orographic structure of the mountain systems on which the butterfly species have persisted until today. This genetic pattern (in combination with findings derived from Climate Envelope Modelling, see Habel et al. 2010, 2011a, and chapter IX) let us assume that the species switched from its panmictic distribution over the Central European lowland during the cold phases to higher elevations during the warming of the postglacial period. These mountain systems of Western Europe (ranging from the Pyrenees, over the Massif Central, the Morvan Mountains, Jura, Vosges, Ardennes and Eifel to the Westerwald) provide “islands” of suitable climatic conditions for cold-adapted species like *L. helle* during the warm climatic conditions of the Postglacial.

Furthermore, these areas are geographically isolated from each other in most cases. Therefore, after retreating to these mountain systems, gene flow among them was strongly restricted or even completely interrupted, and each isolated population group evolved a strongly differentiated gene pool due to the stringent isolation over several thousands of years (Fig. 2). The different population groups of these mountain areas are also characterised by a high proportion of private alleles; more than 11% of the total number of microsatellite alleles observed are restricted to a single mountain range (Habel et al. 2010). In addition to these unique alleles and allele frequencies in microsatellites and, to a lesser degree, allozymes, morphological characters distinguish the populations of each of these mountain areas so that *L. helle* was split into nine subspecies (Meyer 1982, further details in chapter VIII). Taken together, these data indicate that the local populations of *L. helle* represent fairly recently isolated population units with distinct evolutionary trajectories, which needs to be taken into account when developing conservation strategies for the species.

CONCLUSION

Based on three molecular markers, we derived the following biogeographic scenario for *L. helle*: (i) the species was widespread and represented an interconnected network of populations in the periglacial part of Europe during the cold phases mirrored by the lack of genetic differentiation of mitochondrial DNA sequences; (ii) postglacial northwards expansion to Fennoscandia as highlighted by microsatellite frequencies and allele elimination in the wake of this colonisation; (iii) extinction of *L. helle* over major parts of the Central European lowlands; and (iv) escape to the higher elevations of mountain systems of Central Europe with subsequent distinct evolutionary processes on mountain archipelagos, as identified by allozymes, microsatellites and morphological data. These dynamics are further supported by results of climate and land-use modelling, supporting a large expansion of cool climatic conditions during the LGM (Habel et al. 2010) followed by strong retraction of the suitable climate niche. This is accompanied

by the restriction of cool and moist conditions and respective habitat features to higher elevations over Central Europe and the more northern and north-eastern parts of the continent.

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How genetics can contribute to conservation

Aline Finger^{1*} & Chris Kettle²

¹Genetics and Conservation, Royal Botanic Garden Edinburgh, UK-Edinburgh EH3 5LR,
United Kingdom

²ETH Zürich, Institute of Terrestrial Ecosystems, Ecosystem Management, CH-8092
Zürich, Switzerland

*Corresponding author: Aline Finger, Genetics and Conservation, Royal Botanic Garden
Edinburgh, 20a Inverleith Row, UK-Edinburgh EH3 5LR, United Kingdom,
E-Mail: a.finger@rbge.ac.uk

Compared to ecological field studies, molecular ecology can not only reveal important characteristics of a species' genetic constitution, but also its evolutionary history, demography, dispersal, kinship and mating system (Avice 2004). Conservation Genetics is an interdisciplinary science that applies molecular techniques and genetic theory to explore threats to natural populations associated with small population sizes and/or limited gene flow (e.g. population isolation, genetic drift, genetic bottlenecks, inbreeding or inbreeding depression) to reduce the risk of population or species extinction (Frankham et al. 2004).

The motivation for such research is based on theoretical and empirical evidence that populations which have been reduced in size are vulnerable to a reduction in genetic diversity, population isolation and Allee effects (Berec et al. 2007), which can lead to an increase in mating events between close relatives (inbreeding). Elevated inbreeding can be problematic because it increases the chance of deleterious or lethal recessive genes being expressed. These genes normally occur at very low frequency, in their heterozygous form, but are multiplied through a combination of similar genes as individuals or populations become more homozygous (carrying two identical copies of the same allele). This so-called inbreeding depression can negatively affect individual and population fitness (Angeloni et al. 2011). High genetic diversity provides a species with the ability to adapt to environmental changes, which may be crucial for its long-term persistence. Populations with a low genetic diversity are expected to suffer more seriously from diseases, pests and parasites (Spielman et al. 2004).

To quantify genetic diversity and to determine levels of inbreeding, genetic differentiation and gene flow, an array of techniques are available. These either directly or indirectly measure DNA based sequence variation. Only very small amounts of DNA are necessary, which can be extracted non-invasively from small samples of tissue such as hair, faeces or leaves. Different sequences on a particular locus of a DNA molecule result in variants, which can be detected with the help of molecular markers. Neutral molecular

markers (found on non-coding or non-functional DNA sequences) have been widely applied in conservation genetic studies. Below, we describe the most commonly used neutral molecular markers in Conservation Genetics; for further reading see *Avise* (2004).

Nuclear microsatellites, also Short Tandem Repeats (STR) or Simple Sequence Repeats (SSR), consist of short, tandemly repeated sequences of one to six base pairs (most commonly two to four) and are inherited co-dominantly following standard Mendelian traits. Microsatellites are particularly well suited for analysis on small geographical scales, down to the individual level. **Simple Nucleotide Polymorphism**, also SNPs, are alterations of single nucleotides at specific DNA sequences. Due the high numbers of SNPs available and relative ease of finding variable loci, this marker is increasingly used as an alternative to nuclear microsatellites. However, because it requires complete sequence reads for each locus, the high costs of large scale genotyping in non-model organisms remains a drawback. Even though less used nowadays, **Allozymes** have been important in population and conservation genetics. They are variants of enzymes due to allelic differences and can be visualized through protein electrophoresis. Due to a relatively low average heterozygosity, the application of this marker is suitable for geographically broader scales of extant species, at relatively low costs. In plant studies, multilocus DNA marker systems, such as **Amplified Fragment Length Polymorphism**, AFLP, generate banding patterns that are scored for variation. AFLP finds wide application in studies on population genetics, diversity and differentiation. Nevertheless, important information such as inbreeding coefficients cannot be assessed as the marker is dominant. **Next Generation Sequencing** techniques, or simple NGS, which have the capacity to process millions of sequences in parallel, are useful so far as they allow screening large amounts of e.g. microsatellites and SNPs and by that reduce development costs for these markers.

Genetic data provide a powerful tool to analyse population patterns that cannot be detected by ecological field work alone. Yet, in combination with ecological data, molecular markers generate information on complex biological processes, such as gene flow and mating patterns, which enable conservation management of biodiversity to be better informed and more sustainable.

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Isolated and unique: The conservation value of extant populations of *Lycaena helle* at the species' western distribution edge

Jan Christian Habel^{1*}, Aline Finger², Marc Meyer³ & Thomas Schmitt^{4,5}

¹Terrestrial Ecology Research Group, Department of Ecology and Ecosystem Management, Technische Universität München, D-85354 Freising-Weihenstephan, Germany

²Genetics and Conservation, Royal Botanic Garden Edinburgh, Edinburgh EH3 5LR, UK

³D-66706 Kesslingen, Germany

⁴Department of Biogeography, Trier University, D-54286 Trier, Germany

⁵Senckenberg German Entomological Institute, D-15374 Müncheberg, Germany

*Corresponding author: Jan Christian Habel, Terrestrial Ecology Research Group, Department of Ecology and Ecosystem Management, Technische Universität München, Hans-Carl-von-Carlowitz-Platz 2, 85350 Freising-Weihenstephan, Germany, E-Mail: Janchristianhabel@gmx.de

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Abstract

The distribution range of a species consists of a core area and the distribution margin. While local populations often occur in high densities in the centre of distribution, populations at the periphery often exist in low abundances, smaller population sizes and higher population isolation. Such smaller and more isolated populations are characterised by genetic impoverishment, stronger population fluctuations and, additionally, stronger environmental stochasticity for the given species. Consequently, genetic diversity, which is supposed to be negatively correlated with population size, should be lowest at the periphery of a species' distribution range. To determine whether these effects are visible in western peripheral populations of the Violet Copper *Lycaena helle*, molecular genetic techniques determining the degree of genetic diversity and differentiation were applied. Microsatellites and allozymes show strong genetic differentiation according to the orographic structures of the mountain areas to which populations are restricted. Each of these regions contains a high proportion of the entire species' genetic diversity, but also comprises unique alleles restricted to the single mountain region, thus making these populations particularly valuable for conservation. Despite strong population isolation and a significant genetic differentiation among regional *L. helle* populations, their genetic diversities remain relatively high if compared with other butterfly species surviving as local or regional relicts. We conclude that the studied peripheral *L. helle* populations are of high conservation value as they preserve unique alleles contributing to the high genetic diversity of the species as a whole. Despite being geographically and hence genetically

isolated, these populations represent a high genetic diversity and may therefore be able to respond on environmental changes and thus might be able to persist in such a fragmented environment.

GENETIC DIVERSITY FROM THE CORE TO THE MARGIN

Species' abundance strongly varies over a species' distribution range. While core distribution areas are often characterised by high densities of the respective species, these densities decrease to the peripheries (Asher et al. 2001). Furthermore, populations fluctuate more strongly at their distribution margin due to less favourable climatic and ecologic conditions as well as a disturbed population network and thus higher population isolation. Therefore, peripheral populations may be more severely affected by environmental stochasticity than those in core areas (Melbourne and Hastings 2008). This finally may lead to typical population genetic signatures, with highest diversities and lowest differentiation found in the core of a distribution, and comparatively low genetic diversity and strong genetic differentiation at the distribution margin (Fig. 1; cf. Hampe and Petit 2005).

Population isolation, accompanied by small population sizes, has been shown to have a negative long-term impacts on population viability, as it often leads to low genetic diversity and increased inbreeding (i.e. matings between close relatives, as frequently demonstrated in laboratory studies on *Drosophila* species; e.g. Ball et al. 2000; Bijlsma et al. 2000; Reed et al. 2002; Kristensen et al. 2008). The fixation of rare and often even deleterious alleles and the subsequent inbreeding, on the long run, can lead to a reduced

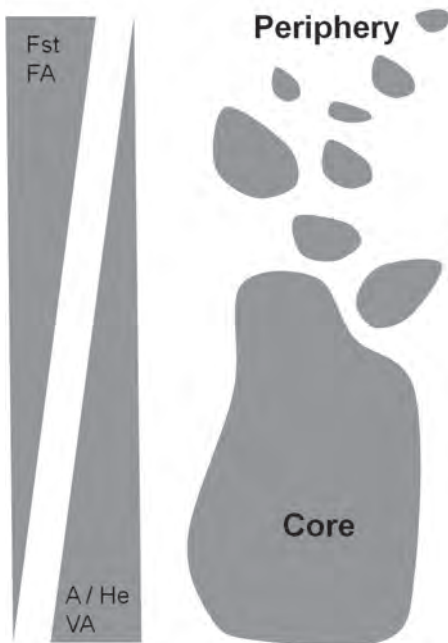


Figure 1: Biology of extant populations (redrawn after Hampe and Petit 2005). Reduced population densities (e.g. declining abundances) of species cause rising genetic differentiation (e.g. F_{ST} values as measure of the genetic variance among local populations) and a decline of genetic diversity (e.g. A , mean number of alleles or H_e , expected heterozygosity); morphological measures (e.g. morphologic variance VA and the deviation among bilateral symmetric structures like fluctuating asymmetry FA) can be used to characterise the condition of populations over a species' distribution range.

individual fitness (Spielman et al. 2004; Frankham 2005) as also has been demonstrated *in situ*, e.g. for the European Adder *Vipera berus* (Madsen et al. 1999, 2004).

In contrast, other examples demonstrate that population isolation and low genetic diversity are not necessarily linked to low fitness or species extinction. For example, a relict subspecies of the Red Apollo butterfly, *Parnassius apollo viningensis*, was nearly driven to extinction some decades ago, but does not show any decrease in viability after habitat restoration, despite being genetically depleted (Habel et al. 2009). This population consists of solely monomorphic individuals in microsatellites and allozymes. This maintenance of viability despite low genetic diversity might be explained by “purging”, a process during which, by chance, low-frequency deleterious alleles are lost and thus cannot be expressed, even if the referred group of individuals is completely homozygote (Frankham et al. 2004). However, a low genetic diversity may impede a species or population to adapt on changing environmental conditions and may therefore be particularly dependant on specific habitat conditions, rendering it vulnerable despite its temporary survival (Habel and Schmitt 2012). In contrast to the described population of the Mosel valley, other molecular studies on *P. apollo* populations, using identical molecular markers for provenances from the Pyrenees and Alps, yielded a certain, but low, extent of polymorphisms (Descimon 1995, Meglecz et al. 2004), demonstrating that the species *per se* has some genetic variability hereby supporting the idea that the case of genetic monomorphisms in *P. apollo vinningensis* is linked to purging.

Generally, high genetic diversity and low degree of inbreeding should enable taxa and populations to persist in a changing world, either caused by climate change or anthropogenic land-use modifications. As populations at the periphery are mostly characterized by stronger genetic differentiation among populations, a lower genetic diversity and a higher morphological variability within populations compared to their core distribution (Chang et al. 2004; Hampe and Petit 2005) (Fig. 1), their long term persistence may depend much more on constant habitat conditions than in the core areas. In the following, we explore whether peripheral populations of *L. helle*, which are restricted to the higher elevations of the Middle mountains of Central Europe at the species’ western distribution edge, are genetically impoverished and whether the populations therefore are at a higher risk of getting extinct.

GENETIC UNIQUENESS ON MOUNTAIN TOPS

The boreo-montane butterfly *L. helle* is adapted to cool and moist habitats such as wet meadows not yet having transgressed a certain threshold of succession (Turlure et al. 2009). The larvae are exclusively feeding on *Polygonum bistorta* in Central Europe (but *Polygonum viviparum* in Fennoscandia, Henriksen and Kreuzer 1982, chapters I, III and IV of this book). The climatic warming and direct habitat modifications by drainage and afforestation of these wet habitats have led to a strong decline of this butterfly during the past decades (Fischer et al. 1999). Hence, *L. helle* is listed in several national and regional Red Lists and the Appendices II and IV of the Natura 2000 directive (Biewald and Nunner 2006).

Especially the western distribution range of *L. helle*, where the species occurs restricted to mountain regions (like the Pyrenees, Massif Central, Jura, Morvan, Vosges, Eifel, Ardennes, Westerwald, for some habitats see Fig. 3), is of particular interest in the light of evolutionary biology, conservation biology and biogeography. During the Würm ice age, the species most probably occurred over major parts of the Central European lowlands not covered by glaciers. The geographically more isolated moist and cool areas at higher altitudes were only populated in the wake of the postglacial warming when the species was forced to retreat to cooler regions (Habel et al. 2010, chapter VI of this book).

Analyses of polymorphic microsatellites and allozymes (details on markers and loci are given in Habel et al. 2008, 2011, for avoiding damages to the populations, only single legs of the individuals were sampled in most cases and individuals were marked (Fig. 4) to avoid resampling of the same individual) evidence strong genetic differentiation between mountain areas, resulting in populations with a mountain-specific genetic make-up (Fig. 2). A large proportion of all alleles detected solely occurs in single mountain ranges (Habel et al. 2010, 2011). Most of this genetic structure is supported by differences obtained from analysis of wing colouration and patterning: Meanwhile, nine subspecies of *L. helle* are distinguished for Central Europe (Meyer 1982, chapter VIII of this book). However, neither data obtained from mitochondrial DNA sequences of the *COI* gene, nor geometric morphometric approaches using wing-vein structures coincide with this classification (see chapters VI and VIII).

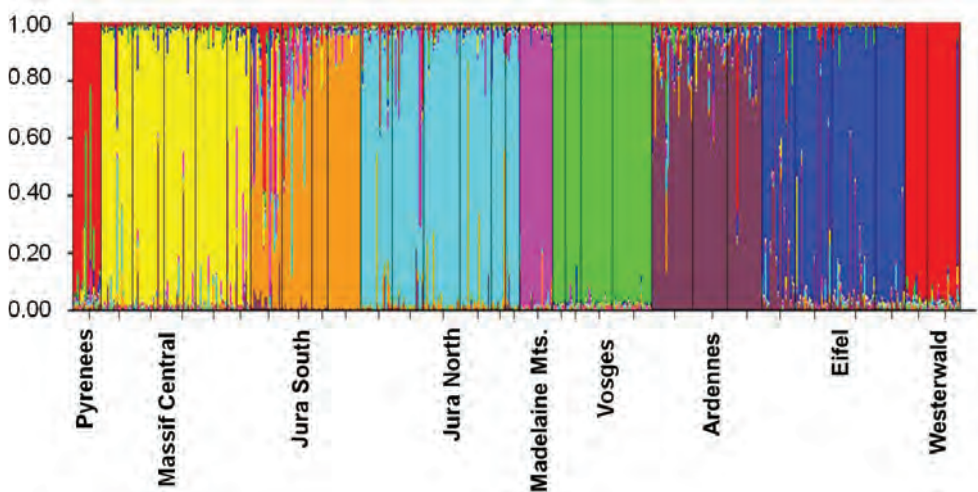


Figure 2: Genetic differentiation of *Lycaena helle* populations analysed using polymorphic microsatellites. Clusters calculated using the programme STRUCTURE (Pritchard et al. 2000) with given $K = 8$ are supported by the highest probability values for the obtained groups. Genetic classifications correspond with the mountain regions in which the population groups were sampled. Each run was repeated ten times to evaluate whether deviations existed among the different runs for a fixed K , and to calculate means and standard deviations. For each run, burn-in and simulation lengths were 100,000 and 300,000, respectively. The calculations were run under the admixture model, with correlated gene frequencies. Data taken from Finger et al. (2009) and Habel et al. (2010, 2011).

The delineation of Evolutionary Significant Units (ESUs) (Moritz 1994) allows to consider the variability within species (based on species' morphology, genetics, physiology and / or behaviour), such as the differentiation observed for some marker systems in *L. belle*. These pieces of information can be considered in action plans for nature conservation. This concept endeavours conserving local adaptations, clinally distributed variation patterns or genetic clades. Following this approach, distinct populations containing genetic uniqueness have to be conserved independently from populations located in the species' distribution core. Thus, a species' entire genetic spectrum can best be conserved by identifying and protecting Conservation Units (Amato et al. 1998). To protect the entire genetic variability of *L. belle*, the conservation of the western genetic groups is of high importance. The high genetic distinctiveness due to population isolation, in addition to potentially differing ecological conditions in the different mountain regions, may have led to local population adaptations, which might increase the relevance to protect local populations to maintain potential local adaptations.

However, transforming this theoretical idea of ESUs into practical actions has been frequently discussed, but rarely realised (Moritz 1994; Amato et al. 1998; Dimick et al. 2001), as the translation from theory into practice is mostly expensive (De Guia and Saitoh 2006). Therefore, Conservation Units were identified only for very few (and mostly large) animal species, e.g. for the Black Rhinoceros *Diceros bicornis* (Brown and Houlden 2000).



Figure 3: Typical habitats of *Lycaena belle* in (a) the Eifel, (b) the Luxembourg and (c) the Belgian Ardennes. The latter shows the habitat with a handful of the Violet Copper's best friends.



Figure 4: The Violet Copper in the Eifel mountains (a) prior and (b) after being sampled, note the black line at the wings margin.

GENETIC DIFFERENTIATION, EVEN WITHIN MOUNTAIN SYSTEMS

The nowadays restricted gene-flow (and thus the lack of intact population networks), even within mountain areas, is being mirrored in the genetic structure of populations. This is particularly visible in the Massif Central where a strong genetic differentiation is apparent on the landscape-level. Moreover, restricted gene-flow has been shown, despite geographically neighbouring populations, for the mountain ranges of the Ardennes and Eifel, which are split into two distinct genetic units (Finger et al. 2009). Warming climate and ongoing habitat destruction may further enhance habitat fragmentation for *L. helle* (see chapter IX). Subsequently, a reduction of local populations and decline in densities is possible, even within mountain areas, maybe further reducing gene-flow rates between and within populations and increasing genetic differentiation.

Although the *L. helle* individuals analysed with microsatellite loci show a strong genetic differentiation among and within mountain ranges, these populations still harbour a high level of genetic diversity. This suggests that a high level of genetic diversity could be maintained despite strong population isolation and that the species, to a certain extent, may be adapted to survive in small populations. In contrast, other butterfly species, which similarly exist in small and geographically isolated populations, have been shown to have much lower levels of genetic diversity, like *Maculinea alcon* (Gadeberg and Boomsma 1997), *Speyeria nokomis apacheana* (Britten et al. 1994), *Euphydryas gillettii* (Debinski 1994), the Zygaenid moth *Aglaope infaustra* (Schmitt and Seitz 2004) and the Red Apollo *Parnassius apollo vinningensis* (Habel et al. 2009). We assume that the contemporary high genetic diversity found in these relict *L. helle* populations may be caused by intact population networks within single mountains archipelagos. Indeed, it could be shown that species, which formerly occurred in large population networks and suffered rapid habitat changes within short periods of time, have lost a high proportion of their former genetic variability (like *Zygaena loti* and *Zygaena viciae*, Habel et al. 2012a, b).

FRAGMENTED HABITATS *VERSUS* HABITAT FRAGMENTATION

The intensity of fragmentation effects on population diversity and viability may strongly depend on the time-scale in which a habitat network is transformed into isolated and scattered habitat patches. While rapid habitat destructions may evoke random bottleneck effects and thus may severely influence the genetic variability and hence population fitness, a long-term or natural population isolation over thousands of years may give species enough time to adapt to persist in small populations, e.g. by purging deleterious alleles or ability for long-distance gene-flow (Habel and Schmitt 2012).

To assess the influence of genetic variability on individual fitness, morphologic analyses may give comprehensive information. The phenotypic plasticity (morphological variability among individuals) is assumed to be positively correlated with genetic diversity in the majority of cases, while the level of bilateral symmetries within individuals (e.g. patterns of wing-veins of butterflies and their deviation from bilateral symmetries, i.e. fluctuating asymmetry) is thought to be negatively correlated with genetic diversity (Hoelzel et al. 2002; Zachos et al. 2007). In this respect, we can analyse temporal and spatial sample designs: Individuals of populations located in the core area of their distribution range or populations being part of large networks often show higher morphological variability than individuals at the periphery of the distribution (Agarwal 2001). These measurements are therefore often used to assess species' and populations' fitness (Lens et al. 1999, 2001; Lens and van Dongen 2001; Agrawal 2001; Alibert and Affray 2003). These analyses allow the detection of potential inbreeding depressions due to environmental stress or anthropogenic habitat transformation – when integrating individuals, which were collected representing the pre- and post-fragmentation situation (cf. Vrijenhoek and Lerman 1982; Leary et al. 1984; Hoelzel et al. 2002; Leamy and Klingenberg 2005). Wing-structures (based on landmarks of the wing-vein intersections) of *L. helle* individuals from the Westerwald were analysed covering a large time-span; the results indicate an strong increase in the level of fluctuating asymmetry in this area (for details see chapter VIII). This increase can be interpreted as a response on recent increase in habitat fragmentation and thus rising environmental stress. This could be used as an early-warning system (see Lens et al. 1999, 2001) for this species, which might go extinct in the near future in the wake of ongoing habitat fragmentation, and a decrease in habitat quality in the remaining remnant patches (see chapter XII).

CONCLUSION

One of the main aims of nature conservation is to protect the ecological adaptability and thus the evolutionary potential of species to cope with future environmental dynamics. To conserve the main proportion of a species' genetic variability, populations incorporating unique genetic traits, such as populations located in isolated peripheral parts of species' distribution ranges, should be given as much conservation priority as the larger core area populations. Peripheral populations are of high importance particularly through the exist-

ence of unique alleles, which may be the starting-point for new inter- and intraspecific evolution and potential adaptations (Lesica and Allendorf 1995; Crandall et al. 2000; Steinecke et al. 2002). These highly isolated and often small populations located at the peripheries are not automatically affected by losses of genetic diversity, and thus can be vital *per se*, as demonstrated by the high genetic diversity level observed for *L. helle*. However, the evaluation of local populations is only reasonable against the background of a range of information, such as the species' phylogeography, further historical range shifts (Avise 2009), the knowledge of the species' ecology and also the use of models to create scenarios of potential future distributions patterns and ongoing trends.

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Fluctuating asymmetry and morphological approaches in ecology and evolutionary biology

Frank E. Zachos* & Katharina Spreitzer

Mammal Collection, Natural History Museum Vienna, A-1010 Vienna, Austria

*Corresponding author: Frank E. Zachos, Mammal Collection, Natural History Museum Vienna, Burgring 7, A-1010 Vienna, Austria, E-Mail: frank.zachos@nhm-wien.ac.at

Just like molecular markers can be used to tackle evolutionary and population biological topics (resulting in evolutionary and population genetics), morphological approaches are also available and have been applied long before the molecular revolution of the late 20th century. The classical approach that comes to mind is the morphometric analysis of individuals (or parts thereof, like for example skulls or insect wings). These data can be used not only to characterise individuals but also populations, and various multivariate statistical algorithms can be applied on morphometric datasets to infer population differentiation, trends in size and shape and (phylogenetic) relationships among taxa or operational taxonomic units. Morphometric data, i.e. length measurements, can be produced „classically“ with calipers or by means of setting landmarks on standardised scans or photographs that are subsequently analysed with appropriate software programmes. In the wake of the ever-increasing power of (personal) computers and the concomitant possibilities of visualising shape and shape differences, so-called geometric morphometrics has become more and more popular.

While there is a restricted technical definition of geometric morphometrics (based on Kendall's shape space and Procrustes distances), in a more general sense, geometric morphometrics is „the class of morphometric methods that preserve complete information about the relative spatial arrangements of the data throughout an analysis“, allowing for the „visualization of group and individual differences, sample variation, and other results in the space of the original specimens“ (Slice et al. 2009). Following Kendall, shape in geometric morphometrics is defined as „all the geometric information that remains when location, scale and rotational effects are filtered out from an object“ (Kendall 1977). Geometric morphometrics has recently been applied to a vast range of ecological and evolutionary questions, including population differentiation, taxonomic discrimination and speciation processes in adaptive radiations. It is important to note, however, that morphological population biology is not restricted to morphometric (continuous) characters, but can also be done based on non-metric or meristic (discrete) characters (see

Ansorge 2001 for a detailed review). Typical non-metric characters analysed are bristle number in insects, nerve or blood vessel foramina in vertebrate skulls, dental occlusal variants in mammal teeth or fin ray number in fish.

Both metric and non-metric characters can also be used to assess levels of asymmetry in individuals (and populations). Asymmetry research in biology goes back a long way, with the first comprehensive treatment being the seminal book of Wilhelm Ludwig in 1932. Three different types of asymmetry are distinguished:

1. *Fluctuating asymmetry* (FA): Right-left differences due to random deviations from bilateral symmetry (see also chapter 8).
2. *Directional asymmetry*: Right-left differences due to the stronger development of one side (either always the right or always the left).
3. *Antisymmetry*: Right-left differences due to stronger development of one side, but variability of the side affected.

Fluctuating asymmetry is the rule rather than the exception in bilaterally symmetric taxa (Bilateria) – as you will easily find upon looking in a mirror. The reason is that our developmental genetic programme has to be realised against a variety of environmental influences (temperature, pressure etc.), and non-equal allocation of resources to both sides of the symmetry plane is inevitable. Since this will happen at random, it will sometimes be the right and sometimes the left side that is more developed (longer, heavier, more bristles or foramina present etc.). As a consequence, the distribution of right-left differences ($R - L$) for metric traits will be normal with a mean of zero. Directional asymmetry occurs when one of the two sides is always more pronounced than the other, again causing a normal distribution for metric $R - L$, but this time with a mean unequal to zero. This condition is rarer than FA, but still found quite often. Well-known examples include snail shell coiling (most of them are dextral or right-handedly coiled); the mammalian heart (where the left side is much larger); and the skulls of dolphins where the premaxillary, maxillary and nasal bones (among others) are bigger on the right side than on the left. Antisymmetry, on the contrary, is rather rare, and since this condition always implies asymmetry, but no bias as to which side shows greater development, the concomitant distribution is typically bimodal. A classical example of antisymmetry are male fiddler crabs (*Uca* sp.) which have a small and a huge claw (but both can be on either side).

Usually, ecologists and evolutionary biologists are interested in FA rather than the alternative types of asymmetry, and a statistical test of whether the asymmetry under study really is FA is indispensable as is the quantification of measurement error because the differences between the right and left sides are usually very small compared to trait size (after all, the trait is genetically „supposed“ to be bilaterally symmetric). Therefore, it is important to make sure that the absolute right-left difference ($|R-L|$) is still larger than measurement error. For more on the statistical treatment of asymmetry data and different indices of FA, see Palmer and Strobeck (1986, 2003).

The rationale behind the fascination with FA among ecologists and evolutionists is the following: If bilateral symmetry is the ideal condition (as coded for in the genome), then FA is an „unwanted“ deviation from it, and it should be a sign of quality for individuals if they manage to build a bilaterally symmetric phenotype in the face of environmental influences during development. The closer an individual gets to symmetry, the more successful it must be in compensating and buffering external disturbances. FA is thus a phenotypic manifestation of developmental homeostasis or developmental stability and often used as a proxy for fitness. Consequently, a vast literature deals with the correlation of FA and various other fitness markers, and indeed it has often been found that FA is negatively related to attractiveness and mating success, family size (lifetime reproduction success), predation success and predator avoidance, susceptibility to diseases and parasites etc. (for a review see Møller and Swaddle 1997). Consequently, FA has repeatedly been suggested as a mean of assessing stress-induced threats and decrease in fitness (for an example with an endangered bird and further references see Lens et al. 2002), and management actions have been shown to positively impact levels of FA (estimated based on metric wing traits) in Apollo butterflies (*Parnassius apollo*) (Schmeller et al. 2011).

It has also been a longstanding hypothesis (going back to the 1950s) that FA is correlated negatively with genetic variability such that more heterozygous individuals (and populations consisting of them) show more symmetric phenotypes than homozygous individuals/populations. In principle, higher genetic variability is believed to result in an increased biochemical flexibility at the phenotypic level and may thus foster developmental canalisation (in the face of environmental perturbations), leading to morphologically more symmetric phenotypes. The underlying genetic cause may be one or a combination of the following three:

1. The (enzyme) loci analysed for heterozygosity are responsible themselves (key loci);
2. Loci in linkage disequilibrium with those analysed influence levels of FA;
3. Overall heterozygosity across the genome is negatively correlated with FA.

If the last hypothesis is correct, then heterozygosity also at neutral loci (e.g. microsatellites) distributed across the genome will show correlations with FA levels, provided that the number and location of the loci analysed is representative of the overall genome. Precisely this point has been a matter of contention as analyses are usually performed with low numbers of markers, most often around 10–20. This said, there are still a number of convincing findings relating genetic variability with the level of FA in individuals and populations (see chapter 5 of Møller and Swaddle 1997 as well as Zachos et al. 2007 and references therein). Nonetheless, this interesting question is far from being settled, and it may well be that genetic factors, although present, play a relatively minor role in deviations from FA in many cases. Trait choice (for example metric vs non-metric characters) and taxonomic differences (homoiotherms, particularly placental mammals, have a more stable developmental environment than poikilotherms) may further add to inconsistent results across different study designs.

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Morphologic variability and signals of environmental stress: Wing-shape analyses in the Violet Copper *Lycaena helle*

Jan Christian Habel^{1*}, Sarah Mattern², Marc Meyer³ & Thomas Schmitt^{2,4}

¹Terrestrial Ecology Research Group, Department of Ecology and Ecosystem Management, Technische Universität München, D-85350 Freising-Weihenstephan, Germany

²Department of Biogeography, Trier University, D-54286 Trier, Germany

³D-66706 Perl-Kesslingen, Germany

⁴Senckenberg German Entomological Institute, D-15374 Müncheberg, Germany

*Corresponding author: Jan Christian Habel, Terrestrial Ecology Research Group, Department of Ecology and Ecosystem Management, Technische Universität München, Hans-Carl-von-Carlowitz-Platz 2, 85350 Freising-Weihenstephan, Germany, E-Mail: Janchristianhabel@gmx.de

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Abstract

Population isolation and population size is of central interest in the conservation of species. Relict populations located at the margin of a species' distribution in particular suffer more severely from population fluctuations and environmental stochasticity than populations located in the distribution core. The endangered Violet Copper *Lycaena helle* is a boreo-montan butterfly distributed from Fennoscandia to the Alps. It occurs in often small and isolated relict populations in the Middle Mountains of Europe, but in parallel in large population networks scattered over the northern parts of the Alps including the Jura region. This pattern provides an excellent situation to analyse (i) intraspecific differentiation among local occurrences, (ii) the impact of environmental stress in isolated and small occurrences compared with populations from large networks, and (iii) temporal trends of intraspecific variability in populations from stable environments from the Jura and declining populations from other Middle Mountains. We applied geometric morphometrics of wing-vein structures to distinguish among local populations and to measure within-individual variance. We detected no significant morphologic differentiation among individuals from the Eifel, Westerwald and the Jura. The level of fluctuating asymmetry (deviations from bilateral symmetries from the left and right wing shapes) showed similar levels for both, the relict populations (Eifel and Westerwald) as well as for the population networks from the Jura. However, individuals collected from a geographically isolated and small population from the Eifel (Blankenheim), and a large population network from the Jura show contrasting temporal trends in the morphologic variability among individuals within these local populations:

while the individuals from the Swiss Jura show a stable intraspecific variance over this time period, individuals from the small and isolated Eifel population have suffered under these conditions as signalized by declining morphologic variance. This example evidences the negative impact of small and isolated populations on the intraspecific variability.

INTRODUCTION

Population isolation, population size and habitat quality are of high relevance for the structure and persistence of species' occurrences (Dennis and Eales 1997; Hanski 1998). Large and interconnected populations are often characterised by higher intraspecific variability and viability (Madsen et al. 1999, 2004; Frankham et al. 2001; Allendorf and Luikart 2007) and often survive stochastic population fluctuations, which in invertebrates easily can fluctuate by three orders of magnitude from one generation to the next. Such strong population fluctuations in local populations can easily be compensated by a high frequency of individual exchange with neighbouring populations (Melbourne and Hastings 2008). In contrast to large and interconnected populations, small and isolated ones suffer much stronger under environmental stochasticity and subsequent population fluctuations. Therefore, such populations are at a much higher risk of extinction as fluctuations are not buffered by recolonizations of individuals from adjoining populations (Melbourne and Hastings 2008). Apart from complete losses of local populations, such processes may also cause severe reductions in intraspecific variability, with negative consequences for the individual fitness due to reduced adaptability (Allendorf and Luikart 2007).

Molecular characters have frequently been used to analyse population structures (differentiation and variability) and to assess the recent status of small and isolated populations (Keller and Largiadèr 2003; Keller et al. 2004, 2005). Genetic data have frequently been combined with morphometric measures to cover both, genotypic and phenotypic effects (e.g. Batori et al. 2012; Habel et al. 2012; Hollos et al. 2012). In geometric morphometrics, two measures are of central interest: the variance among individuals (i.e. the intraspecific variability) within one cohort (i.e. population), and within the same individual between both sides (e.g. left and right wing side in the case of butterflies). High morphologic variability among individuals within a population is often interpreted as a signal of high viability. In contrast, high variability within single individuals, i.e. the deviation from bilateral symmetries like between the left and right wing-vein structure (termed fluctuating asymmetry), is frequently assumed as a signal of environmental stress (Lens et al. 1999, 2002) (further details are given in Box V). In consequence, high degrees of fluctuating asymmetry are often found in small and inbred populations (e.g. Hoelzel et al. 2002).

Therefore, geometric morphometrics are a useful tool for analysing populations of the endangered Violet Copper *Lycaena helle*, a boreo-montane butterfly species. In Europe, this species is restricted to higher elevations of the Middle Mountains of Central and Western Europe, where the species is split into various mountain-specific subspecies,

each of them restricted to a single mountain range (Meyer 1981). However, the butterfly also occurs in the northern Alps and eastern Pyrenees as well as in the northeastern European plains and at lower elevations in Fennoscandia in large population networks (see chapters I, II and VI in this book). Its specific habitat requirements, as cool and moist microclimatic conditions (see chapter IX), specific habitat structures and the need of a specific food-plant, *Polygonum bistorta* (*Polygonum viviparum* in Fennoscandia) (further details on the ecological demands of *L. helle* are given in chapter I, II, III, IV, XI, XII), have led to this patchy distribution over Europe, with large population networks in the northern Alps, but small relict populations restricted to mountain massifs in the Middle Mountains, today forming distinct genetic clusters (Finger et al. 2009; Habel et al. 2010, 2011a, 2011b). Recent habitat deterioration (especially in these archipelago-like Middle Mountain exclaves) in combination with climate warming have led to a severe population decline and to the classification of this taxa being endangered in various Red Lists (Binot-Hafke et al. 2011); therefore, it can be found on the Appendix II and IV of the Habitats Directive of Europe (Biewald and Nunner 2006).

The pattern of large population networks in the Alps *versus* small population remnants in the Middle Mountains makes this species an ideal model organism to study effects of long-term and large-scale population patchiness and recent environmental stress on an intraspecific level. We measured wing-vein structures of individuals from *L. helle* collected from three regions (11 populations) with contrasting population situations and histories: individuals from relict populations from two island regions, the Eifel and the adjoining Westerwald in Central Europe, and individuals from one large population network from the Swiss Jura. We used landmarks on the wings to calculate the intraspecific variance among areas, among populations within areas, among individuals within populations, and within single individuals (fluctuating asymmetry). Hereby, we aim to understand the influence of the different population histories on the morphologic variability on these different levels and the effect of the ongoing habitat (i.e. population) decline on the morphologic variability in single individuals. The results allow for the elaboration of conservation strategies.

MATERIAL AND METHODS

The study species

The Violet Copper was not described by Carl von Linné although it was not rare in Sweden by that time. The first valid name given to this taxon is *Papilio helle* (Denis and Schiffermüller 1775). The *locus typicus* is stored in a butterfly collection in Saxony, Eastern Germany (and not Vienna – as erroneously stated by many authors, Lukhtanov and Lukhtanov 1994, Huemer 2004). Since the first taxonomic description, the name of *L. helle* changed several times from author to author (Table 1a). The long struggle for the “right” name for this taxon began with Staudinger in 1871, who selected the name “*helle*”

Table 1: Overview on names of subspecies and variations described for the Violet Copper *Lycaena helle*.A) Different names of *Lycaena helle*.

Scientific name	Leg. and year
<i>Papilio helle</i>	Denis & Schiffermüller, 1775
<i>Polyommatus helle</i>	Denis & Schiffermüller, 1775
<i>Polyommata hellé</i>	Godart Lucas, 1834
<i>Lycaena helle</i>	Denis & Schiffermüller, 1775
<i>Lycaena (Helleia) helle</i>	Denis & Schiffermüller, 1775
<i>Helleia helle</i>	Denis & Schiffermüller, 1775
<i>Papilio hille</i>	Fabricius, 1787
<i>Papilio amphidamas</i>	Esper, 1780
<i>Polyommatus amphidamas</i>	Esper, 1780
<i>Chrysophanus amphidamas</i>	Esper, 1780
<i>Heodes amphidamas</i>	Esper, 1780
<i>Lycaena amphidamas</i>	Esper, 1780
<i>Papilio xanthe</i>	Lang, 1782
<i>Papilio helle</i>	Sensu Borkhausen

B) Names of subspecies, forms and aberrations of *Lycaena helle* in chronological order.

ssp. <i>lapponica</i>	Backhaus, 1876; p. 40; [<i>Polyommatus amphidamas</i>]; locus typicus: Lapponia, leg.: A Kricheldorf
ssp. <i>marchica</i>	Rühl, 1895; p. 210; [<i>Chrysophanus amphidamas</i>]; locus typicus D-Strausberg, Brandenburg
ssp. <i>phintonis</i>	Fruhstorfer, 1910; p. 144; [<i>Chrysophanus amphidamas</i>]; locus typicus: Irkutsk, Siberia
ssp. <i>pyrenaica</i>	Deslandes, 1930; p. 243; [<i>Heodes amphidamas</i>]; locus typicus: F-Porté-Puymorens, Pyrenees
ssp. <i>deslandesi</i>	Hemming, 1932; p. 29; [<i>Lycaena amphidamas</i>]; locus typicus identic to ssp. <i>pyrenaica</i>)
ssp. <i>leonia</i>	Beuret, 1936; p. 272; [<i>Lycaena (Heodes) amphidamas</i>]; locus typicus: CH-Tramelan
ssp. <i>arvernica</i>	Bernardi & De Lesse, 1952; p. [203] 211; [<i>Lycaena helle</i>]; locus typicus: F-Super-Besse, Vallée de Chaudefour, Massif Central
ssp. <i>hellesimilis</i>	Beuret, 1952; p. 101; [<i>Lycaena (Helleia) helle</i>]; locus typicus: CH-Meggen
ssp. <i>magdalenae</i>	Guérin, 1959; p. 88; [<i>Lycaena helle</i>]; locus typicus: F-Monts de la Madeleine
ssp. <i>perrettei</i>	Weiss, 1977; p. 254; [<i>Lycaena helle</i>]; locus typicus: F- Gérardmer, Vosges
ssp. <i>eneli</i>	Betti, 1977 stat. nov.; p. 92; [<i>Lycaena helle</i>]; locus typicus: F-Lac de Remoray
ssp. <i>arduinnae</i>	Meyer, 1980; p. [131] 133; [<i>Lycaena helle</i>]; locus typicus: L-Hoffelt, Hachiville
var. <i>obscura</i>	Rühl, 1895; p. 220 + 747; locus typicus: Leipzig, D-Saxony
♀ f. <i>parvipuncta</i>	Rühl, 1895; p. 210; [<i>Chrysophanus amphidamas</i>]; locus typicus: D-Strausberg, Brandenburg; Rühl (1895) designated Staudinger as author of this form; he mentioned the same form for the second generation in eastern Germany as well as for the populations in northern Scandinavia.
♀ f. <i>crassipuncta</i>	Rühl, 1895; p. 210; [<i>Chrysophanus amphidamas</i>]; locus typicus: D-Strausberg, Brandenburg
ab. <i>derennei</i>	Lambillion, 1913; p. 127; [<i>Chrysophanus amphidamas</i>]; locus typicus: B-Baraque-Michel

♀ ab. <i>amethystina</i>	Wagner, 1919; p. 158; [<i>Chrysophanus amphydamas</i>]; locus typicus: unknown
♀ ab. <i>supradiscoelongata</i>	Courvoiser, 1921; p. 82; [<i>Chrysophanus amphydamas</i>]; locus typicus: unknown
ab. <i>minor</i>	Derenne, 1925; p. 92; [<i>Chrysophanus amphydamas</i>]; locus typicus: B-Malmédy
ab. <i>striata</i>	Hackray, 1938; p. 201; [<i>Heodes amphydamas</i>]; locus typicus: B-Baraque-Michel. Synonymised by Beuret (1953)
ab. <i>radiata</i>	Nordman, 1939; p. 37; [<i>Chrysophanus amphydamas</i>]; locus typicus: FIN-Etelä-Savo, Pulkasalmi
♀ ab. <i>antelutea</i>	Beuret, 1953; p. 57; [<i>Lycaena (Helleia) helle</i>]; locus typicus: D-Dessau, Saxonia-Anhalt
♀ ab. <i>bicolor</i>	Beuret, 1953; p. 57; [<i>Lycaena (Helleia) helle</i>]; locus typicus: D-Dessau, Saxonia-Anhalt
♀ ab. <i>caeruleolunulata</i>	Beuret, 1953; p. 57; [<i>Lycaena (Helleia) helle</i>]; locus typicus: D-Saxony (?)
♂ ab. <i>tristis</i>	Beuret, 1953; p. 58; [<i>Lycaena (Helleia) helle</i>]; locus typicus: CH-Tramelan (?)
♂ ab. <i>juragloria</i>	Beuret, 1953; p. 58; [<i>Lycaena (Helleia) helle</i>]; locus typicus: CH-Tramelan
♂ ab. <i>reducta</i>	Beuret, 1953; p. 58; [<i>Lycaena (Helleia) helle</i>]; locus typicus: CH-Tramelan

nomen nudum due to a short description of the authors, while the name “*amphydamas*” was well described and figured in colour. This fact induced the use of “*amphydamas*” for many decades. Verity finally summarised the facts in the year 1951 and set this name as a synonym, and raised “*helle*” as the valid name for this taxon, conforming to the rules of the International Commission on Zoological Nomenclature (ICZN).

The Violet Copper *Lycaena helle* today occurs over major parts of the Palearctic region and reaches its western distribution edge over Central Europe (Kudrna et al. 2011). As the butterfly is adapted to cool climatic conditions, its distribution is strongly restricted to higher elevations and thus forms distinct population clusters (Habel et al. 2010). The long-term isolation (at least since the postglacial warming about 10,000 years ago) has led to the evolution of various morphological characteristics, like manifold wing-shape colourings. These were formerly described as many subspecies and variations (Meyer 1982; an overview on subspecies and variations described until today is given in Table 1b). A partial overview on the variation in wing colourations is given in Figure 1.

Data acquisition for geometric morphometrics

For geometric morphometrics, we selected individuals from populations, which were previously collected in high numbers at one specific time-point and at one geographic location. We included the following locations, which we subsequently assigned to three different regions: Swiss Jura (CH-Etang de la Gruère, CH-Tramelan, CH-Pass Gurnigel, CH-Selibühl, CH-Morgins); Eifel mountains (D-Blankenheim, D-Perlenbachtal, D-Simmerath, D-Klengelbach); Westerwald mountains (D-Rabenscheid). Details on each sampling location including geographic coordinates, number of individuals used for analyses and the respective museum collections from which individuals were provided are given in Table 2.



Russia - Altai



Swiss - Bern



Luxemburg - Ardennes

Figure 1: Overview of various colour varieties in *Lycaena belle*.



Germany – Dessauer Moor



Poland

Figure 1 (Continued): Overview of various colour varieties in *Lycaena belle*.



France - Vosges



Germany - Leipzig

Figure 1 (Continued): Overview of various colour varieties in *Lycaena helle*.



France - Gerardmer, Vosges



France - Lac de Remoray, Massif Central

Figure 1 (Continued): Overview of various colour varieties in *Lycaena belle*.



France - Pyrenees



Germany - Bavaria

Figure 1 (Continued): Overview of various colour varieties in *Lycaena helle*.



Germany - Mecklenburg-Western Pomerania



Mongolia



Sweden



Ural

Figure 1 (Continued): Overview of various colour varieties in *Lycaena belle*.

Table 2: Sampling localities for which individuals were analysed. Given are region, locality including GPS coordinates, number of sampled individuals and the respective butterfly collection. Abbreviations: CH = Switzerland, D = Germany, L = Luxembourg, NMB= Natural History Museum Bern, AZL = Aquazoo Löbecke, Düsseldorf, Germany; MNHN = Natural History Museum Luxembourg.

Region	Locality	GPS (N / E)	N	Collection
Jura	CH-Etang de la Gruère	47°14'; 7°2'	22	NHMB
	CH-Tramelan	47°13'; 7°6'	77	NHMB
	CH-Gurnigel	46°43'; 7°26'	20	NHMB
	CH-Selibühl	46°43'; 7°25'	33	NHMB
	CH-Morgins	46°13'; 6°51'	18	NHMB
Eifel	D-Blankenheim	50°26'; 6°39'	94	AZL, MNHN
	D-Perlenbachtal	50°30'; 6°14'	16	AZL, MNHN
	D-Simmerath	50°36'; 6°18'	10	AZL, MNHN
	L-Klengelbachtal	50°8'; 6°4'	66	AZL, MNHN
Westerwald	D-Rabenscheid	50°40'; 8°8'	60	AZL

Digital images were taken from the dorsal wing-side of all butterflies using a digital camera (Canon M1, 50 mm lens) under standardised light conditions. Geometric morphometric techniques based on 16 landmarks (all set by the same person) were used to analyse morphometric patterns in wing-shapes. The shape of the forewing is described by nine homologous landmarks, the shape of the hindwing by seven (Figure 2). To assure the repeatability of these landmarks, we exclusively used wing vein intersections or locations where a wing vein meets the edge of the wing.

For both traits, a tps-file was created using the programme tpsutil (<http://www.life.bio.sunysb.edu/morph/>, accessed 01.10.2012). Landmarks were set with the program tpsdig2 vers. 2.12 (Rohlf 2008; freely available at www.life.bio.sunysb.edu/morph/soft-dataacq.html, accessed 01.10.2012). Measurement errors resulting from imaging and landmark digitising can influence the interpretation of results (Palmer and Strobeck 1986, Klingenberg and McIntyre 1998). Thus, each individual was landmarked twice by the same person (SM) to allow a test for the precision of landmark digitising. In this way, only real trends within the dataset are taken into account, and the influence of noise is minimized (Arnqvist and Martensson 1998). To estimate also the amount of measurement error due to imaging, repeated photos were taken of 25 randomly chosen individuals from the population representing the Westerwald mountains: The measurement error obtained from these two photograph series was further calculated.

Statistics

The program MorphoJ vers. 1.01c (Klingenberg 2008) was used to compute morphological variabilities among populations, among individuals within single populations and between the left and right wing-side of one butterfly individual, as well as the measurement error in wing shapes. As the angle between wings and thorax of the butterflies

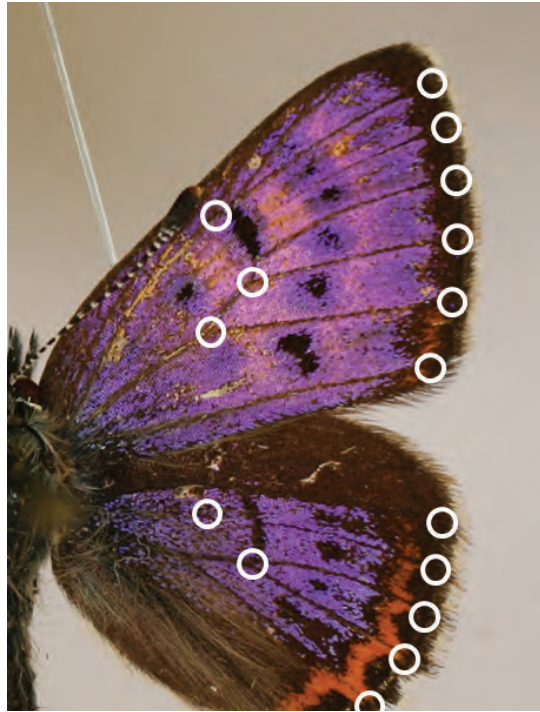


Figure 2: Selected landmarks of the fore- and hind-wing of *Lycaena belle* used for morphometric analyses.

varied considerably, we performed separate analyses for fore- and hindwings. A first generalized least square Procrustes fit analysis was conducted (Klingenberg and McIntyre 1998) including three steps: (1) transformation of all landmark configurations to the centroid, (2) achieving the same centroid size of all objects through scaling all landmark configurations, and (3) rotating the landmark configuration around the centroid until the sum of squared distances between the analogous landmarks is minimized (see Brooksein 1991, Alibert et al. 2001 for details). A Procrustes ANOVA was applied (described in detail in Klingenberg and McIntyre 1998).

Furthermore, changes in morphological variability among individuals over time were tested for the individuals from Tramelan (before and after 1968) and Blankenheim (before and after 1980). For this analysis, individuals were classified into two time cohorts. To test for shape variances in the fore- and hindwing between sexes, among temporal and spatial cohorts, canonical variance analyses (CVA) based on the Procrustes residuals were carried out using the program PAST (Version 1.95; Hammer et al. 2001; freely available at <http://www.folk.uio.no/ohammer/past/download.html>, accessed 01.10.2012).

RESULTS

The measurement errors calculated (i) for individuals photographed twice under identical light conditions and (ii) for digitising the same individual twice by the same person

were negligible when compared with the effects analysed in this study ($< 10\%$ of the variance among individuals and between wing sides). No effect of sex was detected on the shape of both, fore- and hindwings (One-way ANOVA; $P > 0.05$). Therefore, males and females were pooled for further analyses.

The three geographic population groups showed no differentiation among each other (Principal Component Analysis: $P > 0.05$). Even the geographically distant Swiss Jura did not show any differences against the Middle Mountain populations from the Eifel and Westerwald. The level of morphometric variance among individuals (individual) and the level of fluctuating asymmetry (individual \times side) were very similar for individuals from the large and interconnected populations from the Swiss Jura and the individuals from the small and isolated populations located in the Eifel and Westerwald (Table 2).

We found a constant level of morphometric variability among individuals (individual) and within individuals if comparing the two wing sides (individual \times side, i.e. fluctuating asymmetry) for two time cohorts analysed for one population from the Swiss Jura (Tramelan) from 1925–1935 and 1968–1971 (Table 3). In contrast, the morphologic variability of individuals sampled in the Eifel population Blankenheim was higher before 1980 than in individuals sampled later on (Table 4).

Table 3: Procrustes ANOVA based on wing-shape measures for *Lycaena helle* calculated for the three population groups. Given are sums of squares, mean squares and df for dimensionless units of Procrustes distance with the respective P values for the forewing (FW) and the hindwing (HW). * $P \leq 0.05$ ** $P \leq 0.01$ *** $P \leq 0.001$.

Region	Effect	Source	SS	MS $\times 10^{-6}$	df
Jura	Individual	FW	0.7675	349.21***	2198
		HW	0.7451	474.65***	1570
	Side	FW	0.0027	193.53**	14
		HW	0.0016	163.78	10
	Ind \times Side	FW	0.1896	86.28***	2198
		HW	0.2238	142.58***	1570
Eifel	Individual	FW	0.9018	363.94***	2478
		HW	0.6444	368.24***	1750
	Side	FW	0.0047	342.60***	14
		HW	0.0064	643.58***	10
	Ind \times Side	FW	0.1724	69.57***	2478
		HW	0.2777	158.72***	1750
Westerwald	Individual	FW	0.3766	389.92***	966
		HW	0.1950	286.78***	680
	Side	FW	0.0037	266.39***	14
		HW	0.0019	193.48	10
	Ind \times Side	FW	0.0789	81.74***	966
		HW	0.0918	135.08	680

Table 4: Procrustes ANOVA based on wing-shape measures for *Lycaena helle* calculated for the three population groups. Given are sums of squares, mean squares and df for dimensionless units of Procrustes distance with the respective P values for the forewing (FW) and the hindwing (HW). * $P \leq 0.05$ ** $P \leq 0.01$ *** $P \leq 0.001$

Source	Effect	Source	SS	MS $\times 10^{-6}$	df
Tramelan 1925-1935	Individual	FW	0.0651	244.81***	266
		HW	0.0661	348.36***	190
	Side	FW	0.0012	90.07	14
		HW	0.0018	184.85	10
	Ind \times Side	FW	0.0161	60.81***	266
		HW	0.0216	113.73***	190
Tramelan 1968-1971	Individual	FW	0.1627	352.26***	462
		HW	0.1246	377.85***	330
	Side	FW	0.0016	117.33*	14
		HW	0.0019	196.11	10
	Ind \times Side	FW	0.0291	63.08***	462
		HW	0.0393	119.33***	330
Blankenheim until 1980	Individual	FW	0.2389	322.10***	742
		HW	0.2883	554.59***	520
	Side	FW	0.0030	216.53***	14
		HW	0.0069	694.09***	10
	Ind \times Side	FW	0.0497	67.11***	742
		HW	0.1005	193.39***	520
Blankenheim after 1980	Individual	FW	0.1764	331.64***	532
		HW	0.1888	496.99***	380
	Side	FW	0.0009	69.41	14
		HW	0.0028	284.40	10
	Ind \times Side	FW	0.0344	64.71***	532
		HW	0.0642	169.05***	380

DISCUSSION

The obtained morphometric structures based on wing-vein intersections showed no significant morphological differentiation among the three regions, even not among the individuals analysed from the Swiss Jura and the two Middle Mountain regions Eifel and Westerwald. This finding is in conflict with the recent taxonomy, which classifies *L. helle* into various regional and local subspecies (Table 1), including a distinct subspecies for the Alps (including Swiss Jura), for the Eifel, and the Westerwald region (cf. Meyer 1982). The lack of differentiation in wing shapes (wing-vein structures) but regionally diverging wing colouration patterns found among local population groups of *L. helle* goes in line with other studies on butterflies, which showed a strong variation in colouration pattern but identical wing-vein structures. Similar discongruencies (i.e. identical morphometric structures but divergence in colouration patterns) are also found for other animal groups,

as shown for manifold plumage colourations in bird species, accompanied by identical morphometric characters (as tarsus length, bill length, head size, wing length).

The lack of morphologic differentiation among the three population groups of *L. belle* conflicts with the strong genetic differentiation within this species distinguishing distinct mountain specific clusters – also for the Eifel, Westerwald and the Swiss Jura (see chapter VI in this book) as revealed by the analysis of fast-evolving polymorphic microsatellites (Finger et al. 2009, Habel et al. 2010). While the microsatellite markers and the colouration of wings are assumed to be characters evolving very rapidly, the basic structure (i.e. blueprint) of an organism (as the wing venation structure of butterflies) is assumed to evolve rather slowly and has to be classified as a rather conservatively evolving character; hence, the two contrasting responses on the, most probably, postglacial isolation (i.e. differentiation in wing colouration and microsatellites, but identical wing-vein structures) might be due to the, in evolutionary time frames, rather recent vicariance event allowing for genetic drift effects and the evolution of some new mountain-endemic alleles in microsatellites as well as the diversification of wing colouration patterns, but being too recent for a significant differentiation of wing-vein structures among mountain systems.

The morphologic variation found within individuals of the three geographic groups indicate similar mean deviations in bilateral symmetries (FA) (i.e. differences in the wing-shape between the right and the left wing) for the small and isolated populations (in the two population groups from the Middle Mountains Eifel and Westerwald) as well as for the large and interconnected population networks from the Swiss Jura. This finding contradicts the general assumption that the level of fluctuating asymmetry is interpreted as an indicator for environmental stress (which is assumed to be particularly high in small and geographically isolated populations, like our two groups in the Eifel and Westerwald), frequently shown for various species, as butterflies (Adamski and Witowski 2002), birds (Lens et al. 2002) and mammals (Hoelzel et al. 2002, Zachos et al. 2007). However, other studies also revealed that species existing in small and isolated populations since long not necessarily must suffer under the prevailing conditions commonly being interpreted as environmental stress (low population sizes, lack of a population network), as long as resources needed by the species are present. Thus, high viability despite survival in small and isolated populations was recently shown for the local relict populations of a local subspecies of the Red Apollo, *Parnassius apollo vinningensis*; geometric morphometrics showed that individuals do neither show a higher level of fluctuating asymmetry in this small relict population than individuals from large population networks from the Jura, nor an increase in the level of fluctuating asymmetry over time (from 1890 until today) despite small population sizes and recent population bottlenecks (Habel et al. 2012).

In contrast to the similar level of fluctuating asymmetry found over all three populations without considering temporal trends, the morphologic variability among individuals analysed show strong differences when we compare temporal trends: While the morphologic variability among individuals remained rather stable in the Jura during 40 years (1925-1935 to 1968-1971), the morphologic variability in the Eifel population (here Blankenheim) decreased severely over time (by about more than 50%). This loss

in morphological variability in this locality might be the first visible effect of the recent decrease in population sizes and the collapse of the population network in the Eifel region. Therefore, this morphological impoverishment might be a fire signal of first irreversible waves of extinctions.

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Biodiversity Informatics: Quantifying the structure and temporal fluctuations in species' ranges

Dennis Rödder

Zoologisches Forschungsmuseum Alexander Koenig, Adenauerallee 160, D-53113 Bonn,
Germany, E-Mail: d.roedder.zfmk@uni-bonn.de

Scientific collections have a long history of collecting, documenting and preserving biological data. Most specimens housed in museums document scientific studies and provide an important basis for further investigations (Suarez and Tsutsui 2004; Lips 2011). Next to the possibility to answer anatomical, ecological or taxonomic research questions, today information about the exact locality and date where and when a specimen was collected is essential. Such presence data of species is becoming increasingly available through online portals such as the ocean biogeographic information system (OBIS, www.iobis.org) or the global biodiversity information facility (GBIF, www.gbif.org; Graham et al. 2004a). These sources of primary data and facilitated internet-based world-wide access will likely allow dynamic mapping of specimen based distribution patterns in the next future (Jetz et al. 2012). In combination with recent advances in geosciences including climatology and remote sensing, as well as rapid developments in computational capacities, these distribution data sets can be used for large scale analyses of correlates between environmental factors and species' distributions providing insights in a species' niche and ultimately in environmental constraints on the evolution of whole radiations (Kozak et al. 2008).

Based on theoretical considerations, early niche concepts were developed to explain the relationships between a species' distribution and its ecological properties. While some authors focused on resource-related niches operating within smaller geographic scales making them quantifiable via field studies (e.g. Elton 1927), others developed ideas about relationships between species' distributions and niche dimensions comprising non-consumable (termed "scenopoetic") niche axes such as climate variables operating across continental scales (Grinnell 1917). Facing potential impacts of anthropogenic climate change, both theoretical concepts are now again in vital discussion and have been refined and expanded (Soberón and Peterson 2005; Colwell and Rangel 2009; Holt 2009; Rödder and Lötters 2010; Peterson 2011) - last but not least due to the increasing availability of large-scale data sets enabling also a quantification of Grinnellian niches. With the increasing data availability and novel analytical approaches at hand, a steadily

increasing number of studies provide exciting insights into large scale relationships between a species' ecology and its distribution.

Based on the theoretical concepts proposed by Hutchinson (1978), numerous techniques have been developed to predict a species' potential distribution based on approximations of its environmental Grinnellian niche via Ecological Niche Models (ENMs) or Species Distribution Models (SDMs) (e.g. Guisan and Zimmermann 2000; Peterson et al. 2011). Furthermore, although more difficult, some approaches have been suggested how to implement even biotic interactions in these large-scale models (Peterson et al. 2011). Applications of ENMs and SDMs comprise for example risk assessments for invasive species (e.g. Jiménez-Valverde et al. 2011), climate change impacts (e.g. Roberts and Hamann 2012) and spatial conservation planning (e.g. Kremen et al. 2008).

Next to applications in applied sciences, ENMs and SDMs turned out to be powerful tools in basic research fields asking questions from the field of evolutionary biology (e.g. Graham et al. 2004b) as well as in basic ecological research. In combination with palaeo-climatological reconstructions, ENMs and SDMs even allow to project a species' niche across several millennia (Waltari et al. 2007) and, when associated with phylogenetic information, it is now possible to account for evolutionary responses by using phylogenetic regressions and ancestral character reconstruction techniques (Lawing and Polly 2011). Combined with dispersal models, specific evolutionary lineages can be tracked through time (Espíndola et al. 2012), which may be a valuable supplement to phylogeographic analyses (Chan et al. 2011). When projected into the future, such techniques may be of growing interest in times of climate change since they allow deriving spatial explicit conservation implications for genetic lineages. Consequently, recent studies have drawn attention to the potential loss of genetic diversity in numerous species (e.g., Bálint et al. 2011; Rödder and Schulte 2010), including *Lycaena helle* (Habel et al. 2010) (see also Chapter IX).

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Losing genetic uniqueness under global change: the Violet Copper (*Lycaena helle*) in Europe

Youri Martin^{1,2}, Jan Christian Habel^{3*}, Hans Van Dyck² & Nicolas Titeux¹

¹Department Environment and Agro-biotechnologies, Public Research Centre - Gabriel Lippmann, L-4422 Belvaux, Luxembourg

²Behavioural Ecology and Conservation Group, Biodiversity Research Centre, Earth and Life Institute, Université catholique de Louvain (UCL), B-1348 Louvain-la-Neuve, Belgium

³Terrestrial Ecology Research Group, Department of Ecology and Ecosystem Management, Technische Universität München, D-85350 Freising-Weihenstephan, Germany

*Corresponding author: Jan Christian Habel, Terrestrial Ecology Research Group, Department of Ecology and Ecosystem Management, Technische Universität München, Hans-Carl-von-Carlowitz-Platz 2, D-85350 Freising-Weihenstephan, Germany, Email: Janchristianhabel@gmx.de

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Abstract

Global change may strongly affect the geographic distribution of species and induce local extinctions of populations. These changes are expected to induce a loss of intraspecific genetic diversity that may, in turn, induce a decrease in the adaptation potential of the species under changing environmental conditions. Species with limited dispersal abilities, narrow habitat requirements and geographically restricted and isolated distribution patterns, such as the Violet Copper butterfly *Lycaena helle*, are particularly threatened. It is therefore important to evaluate the amount of intraspecific genetic diversity that can be lost in the future to guide proactive conservation strategies able to preserve the adaptation potential of *L. helle*. The restriction of these populations to high-elevation exclaves has led to the evolution of unique morphological and genetic entities, mostly restricted to single mountain massifs. In this study, we examine how future climate and land use changes in Europe are expected to induce range shifts and range contraction in *L. helle* and how these changes may cause extinction of populations and loss of intraspecific genetic uniqueness. We jointly used a species distribution modelling approach and a comprehensive microsatellite dataset to identify the populations of *L. helle* with unique alleles that are expected to be the most threatened with extinction under future global change. We have estimated population extinction and loss of genetic uniqueness under two dispersal assumptions: no dispersal and limited dispersal. Our results indicate that up to half of the currently known populations of *L. helle* are predicted to become extinct by 2080 under the most severe global change scenario. As a consequence, up to one third of the genetic uniqueness is predicted to be lost in Europe.

We found considerable variation in the loss of genetic uniqueness depending on the scenarios used in the projections and on the dispersal assumptions. We suggest that proactive conservation measures should focus on a small number of mountain massifs (e.g. Alps, Jura and Massif Central) in order to maintain the genetic uniqueness of *L. belle* in the future. Our results also highlight the importance of promoting the dispersal of *L. belle* in the future through the management of well-connected networks of suitable habitats.

SPECIES RESPONSE TO GLOBAL CHANGE AND THE EFFECTS ON GENETIC DIVERSITY

Habitat degradation due to land use change in interaction with climate change causes a multitude of environmental pressures that are expected to be the major source of global biodiversity loss in the future (Brook et al. 2008; Mantyka-Pringle et al. 2011). Species may respond to these environmental pressures either by dispersing to avoid unsuitable conditions or by adapting to them, or the combination of both processes (Walther et al. 2002; Davis et al. 2005; Berg et al. 2010; Peñuelas et al. 2013). Hence, species that are able to shift their distribution ranges toward newly suitable conditions and to maintain a high adaptation potential have the highest chances to survive under global change. The level of intraspecific genetic diversity (i.e. the range of genetic variants within all the populations of a species) provides the basis for any evolutionary change that is fundamental for adaptation to environmental change (Coleman et al. 2013; Pauls et al. 2013).

Species range shifts under global change and subsequent extinction of local populations due to lack of suitable conditions are well documented in a variety of plant and animal species, but the consequences of expected range contractions on the intraspecific genetic diversity of the species and on the spatial variation of this genetic diversity remain poorly understood (Collevatti et al. 2011; Bálint et al. 2011; Pauls et al. 2013). Range contractions are expected to induce a loss of intraspecific genetic diversity due to population isolation and extinction (Cobben et al. 2011; Buckley et al. 2012; Arenas et al. 2012). This loss may, in turn, induce a decrease in the adaptation potential of the species in response to global change. It is therefore important to better assess the amount of intraspecific genetic diversity that may be lost in the future and how this diversity may vary in space and over time. This is required if we are to guide proactive conservation strategies able to preserve the adaptation potential of the focal species (Bonin et al. 2007; Funk et al. 2012; D'Amen et al. 2013).

Species distribution models are helpful tools to evaluate how species distributions may shift under global change. These models capture the ecological niche of the species through a statistical link between current species records and environmental conditions (Guisan and Zimmermann 2000; Elith et al. 2010). They may be used to document the spatial pattern of the environmentally suitable areas for a species and may also improve our understanding of the key parameters that influence species distributions. Interestingly, such models can also be projected under scenarios of past or future environmental change to explore the historical distribution of the species or predict its future range

shifts and can be combined with data on intraspecific genetic diversity. This enables to evaluate how future species range shifts may translate into genetic diversity loss (Habel et al. 2011; Bálint et al. 2011; Taubmann et al. 2011; Alsos et al. 2012). Hence, these models may provide valuable information to the conservation management of endangered populations under global change with a focus on species range shifts and intraspecific genetic diversity changes (Scoble and Lowe 2010; Hamann and Aitken 2013).

The integration of genetic diversity data into predictions of future species range shifts is therefore highly recommended to better evaluate the potential of the species to respond to global change (McCallum et al. 2013; Pauls et al. 2013). This is even more urged for species with narrow habitat requirements (e.g. a restricted set of host plants, particular habitat structures or climatic conditions) and/or limited dispersal ability. These species are often associated with geographically restricted distribution patterns and strong population isolation in combination with small population sizes. Hence, they often have a low intraspecific genetic diversity with large genetic differences among populations (i.e. genetic differentiation) (Thuiller et al. 2005; Parmesan 2006; Gibson et al. 2010; Velo-Antón et al. 2013).

The Violet Copper butterfly (*Lycaena helle*) represents this coherence between narrow habitat requirements, limited dispersal ability, geographically restricted distribution pattern and subsequent strong genetic differentiation over varying spatial scales. This boreo-montane butterfly is adapted to cool and moist climatic conditions and thus is of particular conservation concern in the context of recent global change (see Chapter XII, Habel et al. 2011). *L. helle* is only found in the high mountain systems of Europe (Pyrenees, Alps, some parts of the Balkan), in isolated exclaves along the Middle Mountains of Europe (e.g. Massif Central, Vosges, Ardennes, Eifel, Westerwald), and in the lowlands of Eastern and Northern Europe.

Genetic analyses on polymorphic microsatellites and allozymes in *L. helle* revealed a strong genetic differentiation among European mountain populations resulting in the distinction of 13 mountain specific genetic clusters. Populations of *L. helle* in these mountains are considered genetically isolated and most of them enclose many unique alleles that are not found in other mountains (i.e. alleles that are endemic to a single mountain area) (see Chapter VII, Habel et al. 2008, 2010). In contrast to most species associated with geographically isolated populations, narrow habitat requirements and limited dispersal abilities, genetic analyses also indicate that the populations of *L. helle* established in the European mountains have high genetic diversity (see Chapter VII).

This particular genetic structure is the product of large-scale distribution dynamics in the past: *L. helle* showed a wide geographical distribution during the glacial period and covered major parts of the Central European lowlands, but the species experienced a strong restriction to higher altitudes and latitudes in the wake of postglacial warming (Habel et al. 2010). This dynamics has led to long-term disconnection of the remaining mountain populations that evolved separately (see Chapter VI). This resulted in strong morphological and genetic differences among populations (Meyer 1982; Habel et al. 2011).

Populations accounting for a large proportion of intraspecific genetic diversity and populations with unique lineages are both considered as highly valuable conservation units if we aim to preserve the adaptation potential of species under future global change. Therefore, the best strategy to manage such species should consider both genetic diversity and genetic uniqueness (Coleman et al. 2013). In the particular case of *L. helle*, most of the populations in Europe show a high genetic diversity. Therefore, we may argue that proactive conservation strategies should primarily focus on populations that enclose a significant proportion of unique alleles.

In this chapter, we examine how future climate and land use changes in Europe are expected to induce range shifts and range contraction in *L. helle* and how such changes are likely to cause population declines and losses of intraspecific genetic uniqueness. We used a species distribution modelling approach and a genetic dataset based on polymorphic microsatellites to identify the populations of *L. helle* showing unique alleles that are assumed to suffer most under future global change in the near future.

CURRENT AND POTENTIAL FUTURE DISTRIBUTION UNDER CLIMATE AND LAND USE CHANGE SCENARIOS

The loss of genetic uniqueness in *L. helle* was assessed for Western and Central Europe (except Norway due to unavailability of land use change scenarios) covering most of the Western Palaearctic range of the species where its genetic structure is well known (see Chapter VI, Habel et al. 2010, 2011). We combined species presence records from the EU Habitats Directive Article 17 report in 2007 and from previous studies (Habel et al. 2011); in total we obtained 332 presence points (hereafter called populations) over a 10 km resolution grid system to build the distribution models (Fig. 1).

We modelled the current distribution of the species at 10 km resolution using the MAXENT algorithm version 3.3.3e (Elith et al. 2006; Phillips and Dudik 2008). The algorithm captured the ecological niche of the species through a statistical link between the current distribution of *L. helle* populations and a series of environmental variables that reflect the principal habitat requirements and limitations on species growth and survival (Pearson et al. 2004; Luoto and Heikkinen 2008; Heikkinen et al. 2010; Illan et al. 2010). We included three climate variables (growing degree-days above 5°C, mean temperature of the coldest month and water balance), six land use variables (coverage of forest, grassland, built up areas, arable land, permanent crops and other land use types) and one elevation range variable (Mitchell et al. 2004; Dendoncker et al. 2006).

The climate and land use variables were provided with future projections in 2020, 2050 and 2080 according to three integrated socio-economic scenarios developed in the ALARM project (Settele et al. 2005). These scenarios were used to explore the potential distribution of *L. helle* in the future under global change (Martin et al. 2013). The scenarios range from minimum change (SEDG: Sustainable European Development

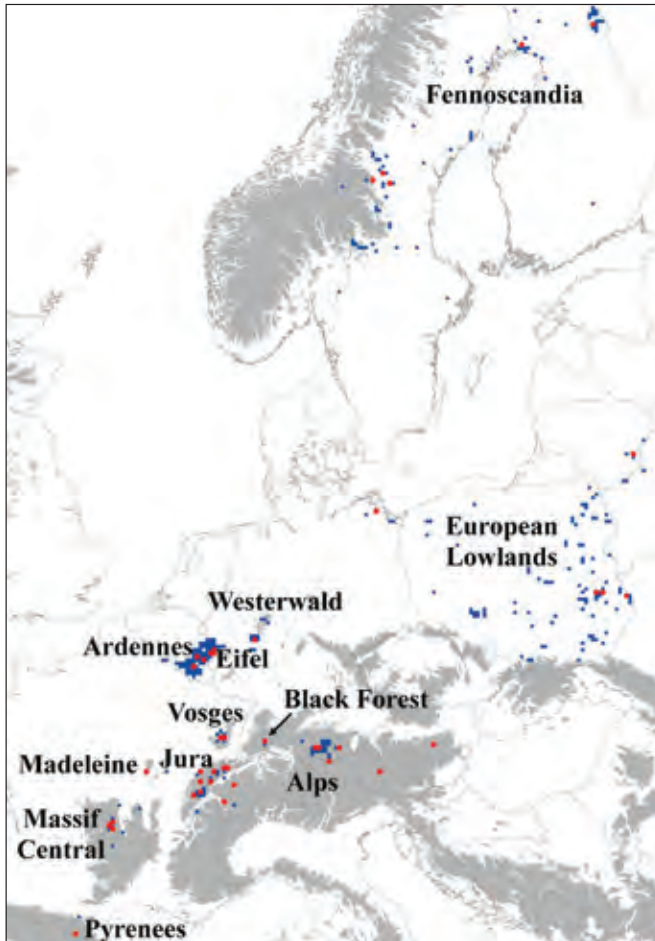


Figure 1: Location of the 332 10 km resolution grid cells with *L. helle* presence records (blue) and 49 populations with genetic samples (red) in Europe. The regions relevant to the genetic differentiation of the species are indicated, most of them being located at an altitude higher than 500 m above sea level (grey areas) (Habel et al. 2010).

Goal) to moderate (BAMBU: Business As Might Be Usual) and maximum changes (GRAS: Growth Applied Strategy) (Spangenberg et al. 2012).

To evaluate the model performance and the robustness of the predictions, the models were built and evaluated ten times with randomly replicated partitioning of the data (10-fold cross validation procedure). Species distribution models were built for the current period (1971–2000) and then projected in 2020, 2050 and 2080 according to the SEDG, BAMBU and GRAS scenarios. Continuous model predictions were reclassified as suitable or unsuitable grid cells for the species based on a threshold that maximizes the sum of sensitivity and specificity of the model (see Liu et al. 2013 for details).

Model performance was good according to model evaluation and over-fitting criteria (training AUC: 0.861 ± 0.003 ; test AUC: 0.840 ± 0.029 ; training omission rate: 0.145 ± 0.033 ; test omission rate: 0.199 ± 0.094). This indicates that the results of the model can be considered reliable and useful for the future projections (Phillips and Dudik 2008; Warren and Seifert 2011).

Mean temperature of the coldest month, forest coverage, growing degree-days above 5°C and grassland coverage were the environmental variables with the highest contribution to explain the distribution of the species (Fig. 2). This is in accordance with the ecology of the species: *L. helle* is a habitat specialist species restricted to open forest ecotones in cool and nutrient-poor grassland habitats (see Chapter XII, Fischer et al. 1999; Finger et al. 2009; Turlure et al. 2009). Hence, the model outputs match the current distribution of *L. helle*, with strong restriction to higher altitude areas (Fig. 3). Furthermore the model projections under global change scenarios provide a relevant overview on the possible shifts in the distribution of *L. helle*, indicating that most of the areas currently suitable for *L. helle* will be gradually lost until 2080 according to the SEDG, BAMBU and GRAS scenarios, except in Northern Europe where environmental conditions will remain or become suitable (Fig. 4).

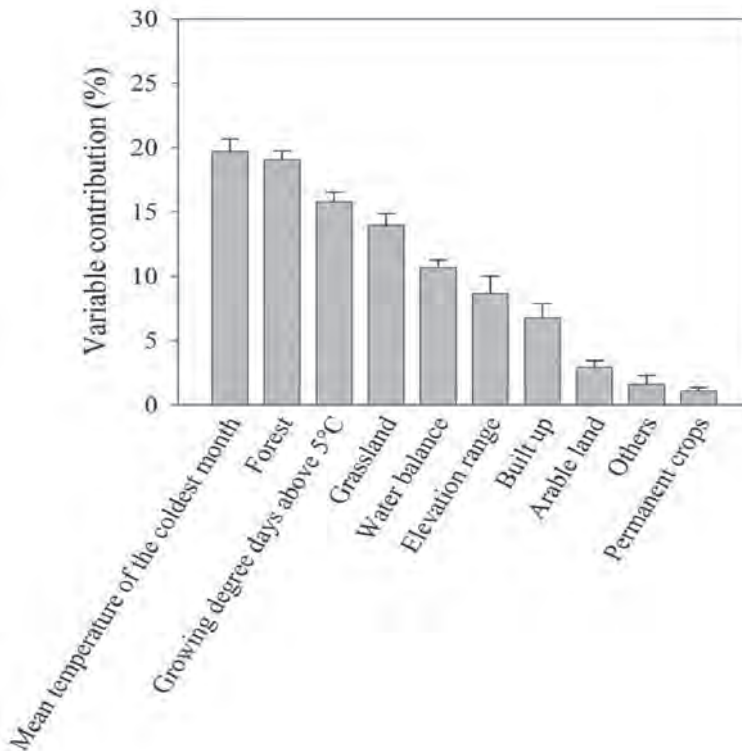


Figure 2: Percent contribution of the environmental variables to the goodness-of-fit of the species distribution models (mean value \pm standard deviation based on ten model replicates).

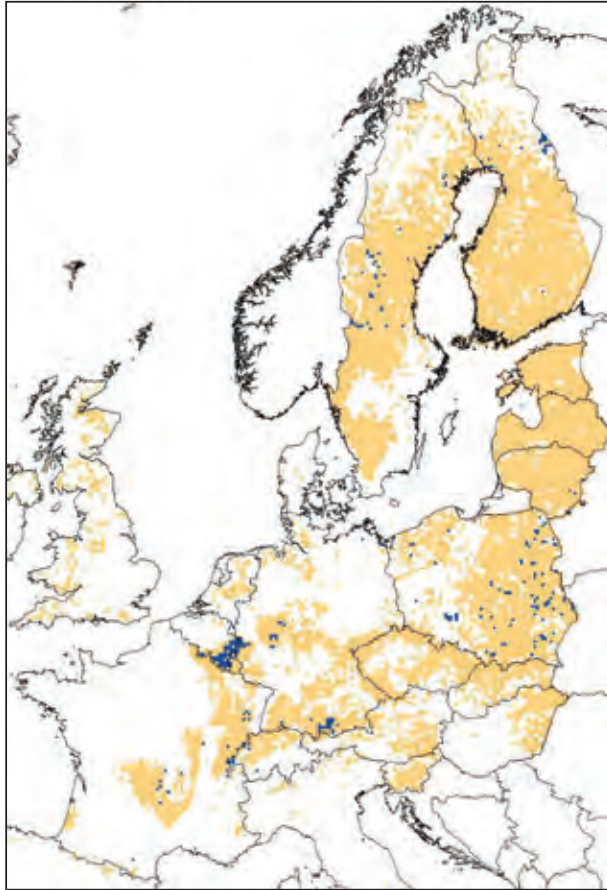


Figure 3: Location of the 332 10 km resolution grid cells with *L. helle* presence records (blue) and predicted range of the species in 1971–2000 (i.e. areas predicted as suitable for the species under current climate and land use conditions) (natural beige).

FUTURE POPULATION PERSISTENCE UNDER DISPERSAL ASSUMPTIONS

Dispersal is a key mechanism for the persistence of populations, and ultimately species, in response to global change (Berg et al. 2010; Stevens et al. 2013), but it is often neglected in species distribution models (Pearson et al. 2006; Thuiller et al. 2008; Barbet-Massin et al. 2011; Buse and Griebeler 2011; De Cáceres and Brotons 2012). Most studies that estimate species range shifts assume unrestricted or no dispersal. These assumptions might be invalid for most of the taxa (Pearson et al. 2006; Thuiller et al. 2008; Barbet-Massin et al. 2011; Buse and Griebeler 2011). Unrestricted dispersal is clearly unrealistic for *L. helle*, but the ‘no dispersal’ assumption may prove to be realistic. *L. helle* is considered to have a very restricted dispersal ability and typically exhibits only rare movements of individuals between well-connected patches (see Chapter 12; Fischer et al. 1999). How-

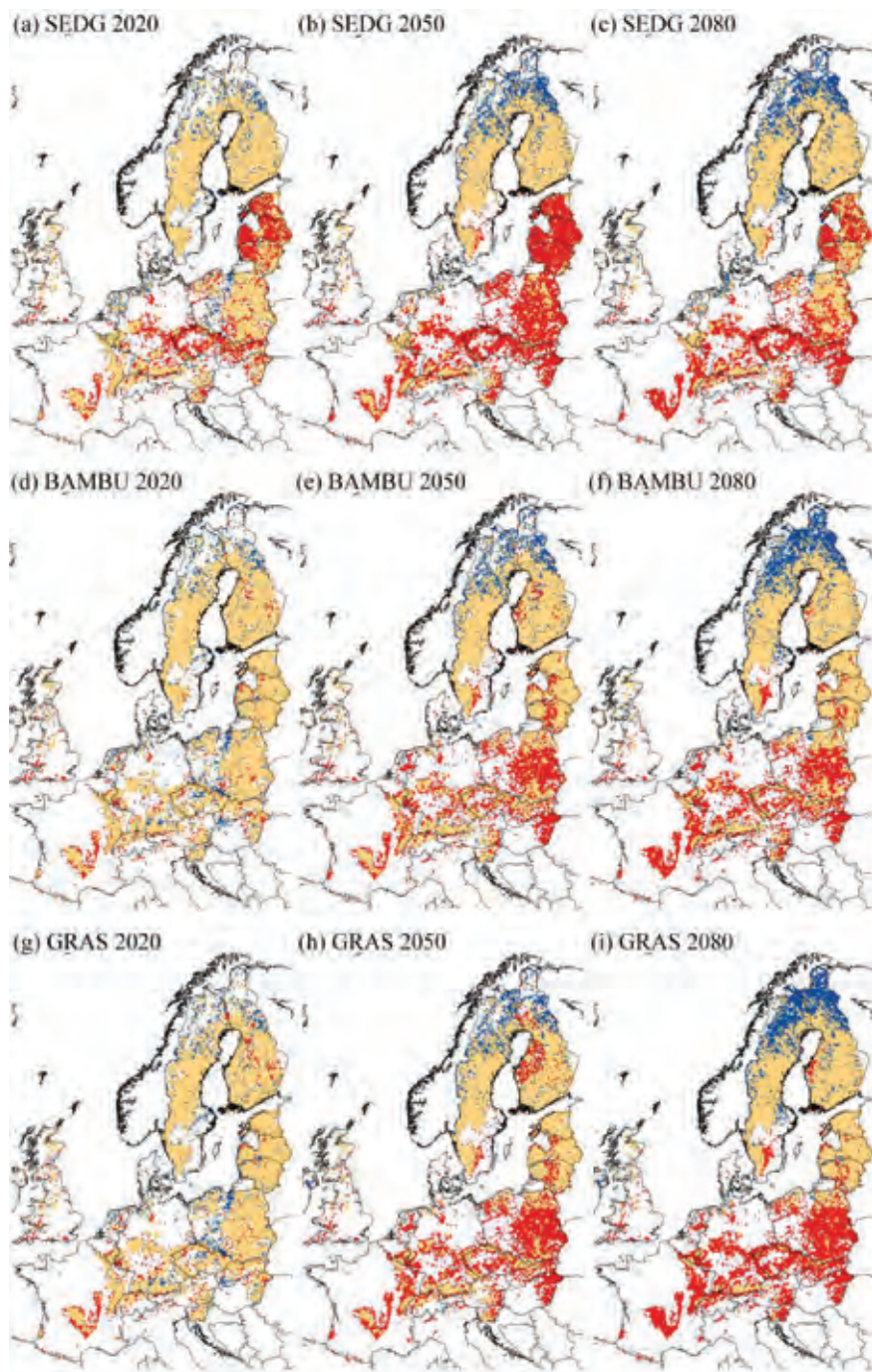


Figure 4: Projected range gain (blue), range loss (red) or unchanged range (natural beige) for *L. belle* at 10 km resolution in Europe according to the SEDG (a-c), BAMBU (d-f) and GRAS (g-i) scenarios in 2020, 2050 and 2080.

ever, Bachelard and Descimon (1999) and Turlure et al. (2013) provided evidence of long-distance dispersal events in this species (see Chapter 4). Hence, we have estimated the future distribution and the population extinction of *L. helle* under two assumptions: no dispersal and limited dispersal.

Under the ‘no dispersal’ assumption, the currently known populations of *L. helle* are expected to be maintained only in 10 km resolution grid cells that will remain suitable over the time frame of the model projections, whereas the others are assumed to become extinct (Fig. 5).

Under the ‘limited dispersal’ assumption, the currently known populations are expected to be maintained if they are established in 10 km grid cells that will remain suitable in the future or if they are able to colonize other suitable grid cells, whereas the others are considered to become extinct. The potentially colonisable grid cells were simulated in space and along the time frame of model projections using the MIGCLIM cellular automaton (Engler et al. 2012). MIGCLIM was applied to the projections of future species distribution in 2020, 2050 and 2080 derived from the modelling procedure. From one time step to the other, populations located in a focal 10 km resolution grid cell that become unsuitable are assumed to be able to disperse to other 10 km resolution grid cells remaining or becoming suitable, but only among the adjacent grid cells. At each time step, populations that are unable to disperse toward suitable grid cells due to the disconnected suitable areas were assumed to become extinct (Fig. 5).

The percentage of populations expected to remain in 2020, 2050 and 2080 under the SEDG, BAMBU and GRAS scenarios, and according to both dispersal assumptions is shown in Figure 6. According to the limited dispersal assumption, the percentage of currently known European populations of *L. helle* that are expected to remain in the future (2080) ranges from 71% (± 3) to 85% (± 2). When assuming no dispersal, this percentage ranges from 48% (± 4) to 62% (± 4). These findings support the general assumption that long-term persistence of local populations is highly dependent on the interconnectivity among suitable areas to guarantee an equilibrium on population extinction and (re) colonisation (Hanski 1998; Akçakaya et al. 2007).

EXPECTED FUTURE LOSS OF GENETIC UNIQUENESS

We analysed five polymorphic microsatellite loci from more than 1000 individuals sampled across 49 populations and located in 13 geographically isolated and genetically distinct European mountains (Fig. 1) (Habel et al. 2008, 2010). Based on this dataset, we identified unique alleles as those that are restricted to a single mountain system (i.e. present in only one of the 13 regions) and counted the number of unique alleles sampled in each population. The potential loss of unique alleles in the future was calculated based on the predicted persistence of the 49 populations with genetic samples under both dispersal assumptions. When a population was predicted to become extinct, we

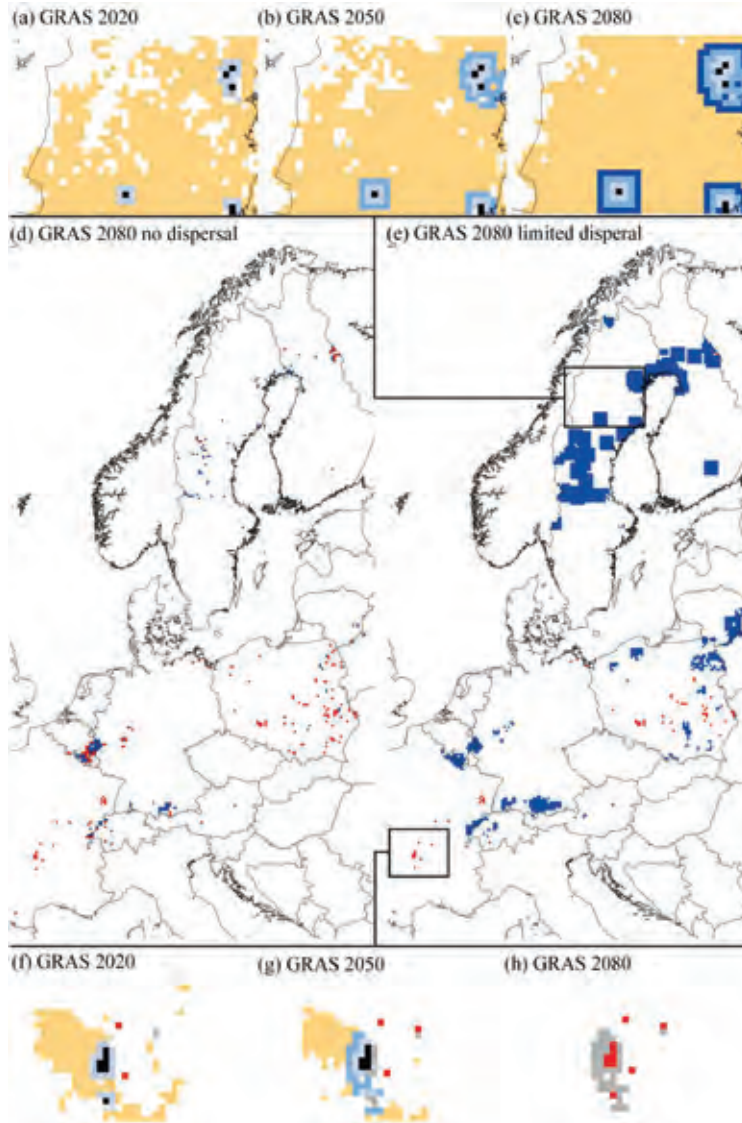


Figure 5: Expected remaining (blue) and extinct (red) populations of *L. helle* in Europe according to no dispersal (d) and limited dispersal (e) assumptions for the GRAS scenarios in 2080. Subsets of the study area in the northern range of the species (a-c) and in the Massif Central (f-h) illustrate details on the potential colonization over the time frame of the projections under the limited dispersal assumption. Currently known populations that will likely be able or unable to disperse towards suitable areas (natural beige) are shown in black or red, respectively. According to the dispersal limitations set in the MIGCLIM automaton, *L. helle* was assumed to be able to colonize suitable areas in adjacent grid cells from one time step to the other, i.e. in light blue areas by 2020, in medium blue areas by 2050 and in dark blue areas by 2080. Grey grid cells show the areas that were suitable and potentially colonized by *L. helle* during the previous time steps but that become unsuitable afterwards.

considered that the unique alleles it enclosed were lost only if these alleles are absent from the remaining populations in the same mountain system.

As expected, the populations in mountain systems were characterised by a high proportion of unique alleles: 30% of the microsatellite alleles (61 out of 205 alleles) were found to be unique. The loss of genetic uniqueness for *L. helle* in Europe under SEDG and BAMBU was predicted to be low according to the limited dispersal assumption with 96% (± 5) and 96% (± 3) of the genetic uniqueness maintained by 2080, respectively. The loss was more important when assuming no dispersal with 72% (± 3) and 78% (± 1) of unique alleles maintained by 2080 according to SEDG and BAMBU scenarios, respectively. The loss of genetic uniqueness was higher under the GRAS scenario, with only 73% (± 6) and 68% (± 4) of genetic uniqueness maintained under limited dispersal and no dispersal assumptions, respectively (Fig. 6).

It is important to note that the predicted persistence of the 49 populations with genetic samples followed the same trajectory as the whole set of currently known populations in Europe (Fig. 6), thereby indicating that the predicted loss of genetic uniqueness based on the 49 populations may be assumed to reflect the European projection. Interestingly, this loss differed considerably among regions: Alps, European Lowlands, North Jura and Massif Central contributed most of the whole loss of genetic uniqueness in Europe under the GRAS scenario in 2080 (see details in Table 1).

CONSERVATION PERSPECTIVES UNDER GLOBAL CHANGE

We have shown that the combined effects of climate and land use changes in Europe will likely induce important range shift and extinction of *L. helle* populations in the future. More than half of the currently known populations are expected to become extinct by 2080 if we consider the most severe global change scenario (GRAS) and no dispersal. Hence, our results support previous studies drawing attention to the conservation status of *L. helle* and urging the implementation of anticipative conservation strategies to accommodate species range shifts to global change (see Chapter VII & XII; Fischer et al. 1999; van Swaay et al. 2010; Habel et al. 2010, 2011).

The conservation of threatened species mainly focuses on populations with high genetic uniqueness because these conservation units are often considered as the most important ones from an evolutionary perspective (Funk et al. 2012; Coleman et al. 2013). Although such populations may enclose unique parts of the intraspecific genetic diversity that could be essential for the adaptation potential of the species, it is also important to maintain populations with a high genetic diversity because they are also more likely to have a high adaptation potential, which could constitute an advantage under changing environmental conditions (Taubmann et al. 2011; Funk et al. 2012; Coleman et al. 2013).

In the particular case of *L. helle*, most of the isolated populations in different mountain systems exhibit a high level of genetic diversity, but only some of them enclose an important proportion of unique alleles (see Chapter VII; Habel et al. 2008, 2010). In the

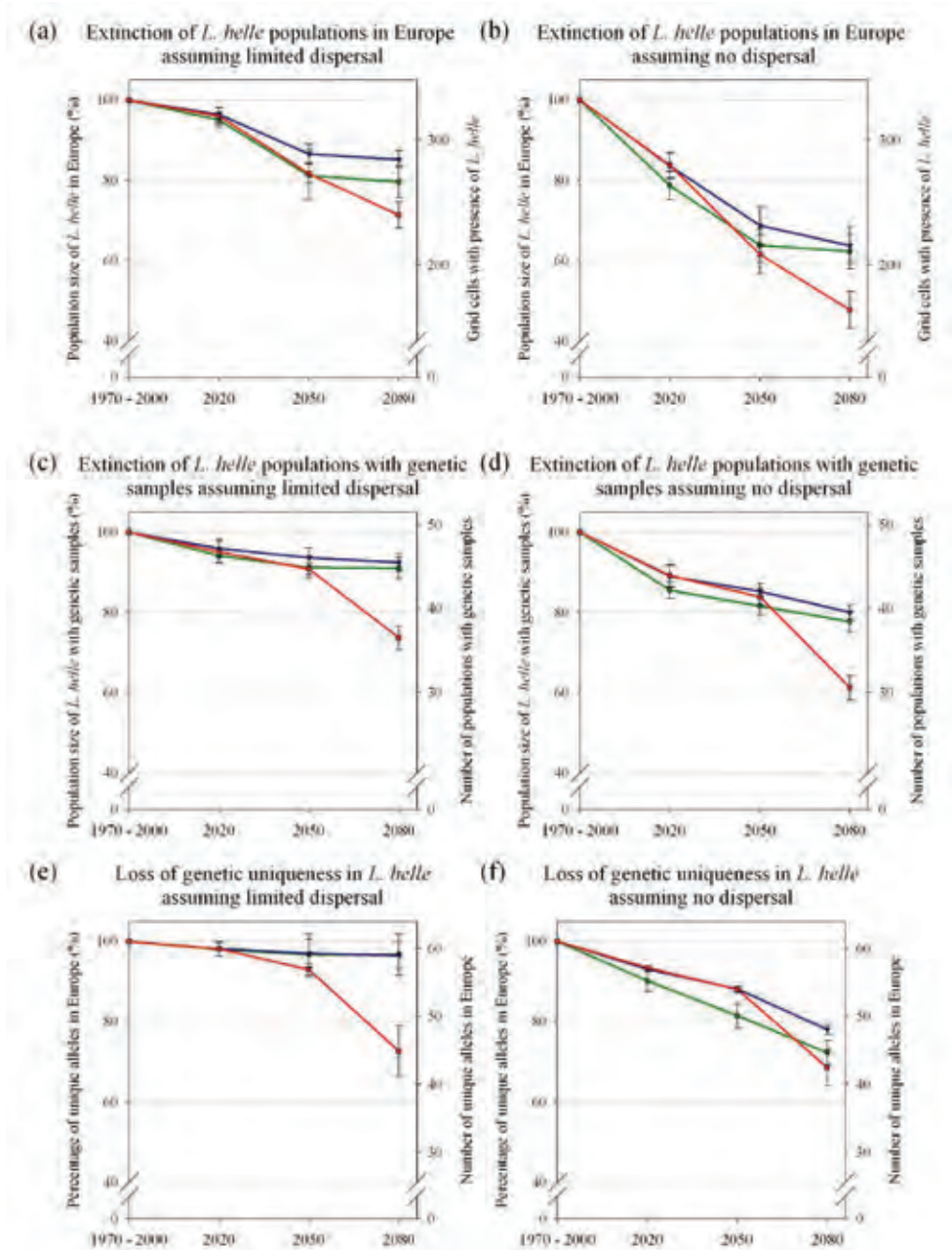


Figure 6: Predicted future population extinction and loss of genetic uniqueness for *L. helle* in Europe. Population extinction in Europe was estimated according to limited dispersal (a) and no dispersal (b) assumptions, for SEDG (green), BAMBU (blue) and GRAS (red) scenarios in 2020, 2050 and 2080. Population extinction was also estimated for the 49 populations with genetic samples only (c, d) to calculate the percentage of unique alleles that are expected to remain in the future (e, f) (see details in Table 1).

Table 1. Expected loss of genetic uniqueness for *L. helix* in each European mountain massif according to the SEDG, BAMBU and GRAS scenarios in 2020, 2050 and 2080 under the limited dispersal and no dispersal assumptions (mean percentage \pm standard deviation based on the 10 model replicates). The number of unique alleles currently enclosed in each mountain massif is indicated between brackets.

Massifs		SEDG			BAMBU			GRAS		
		2020	2050	2080	2020	2050	2080	2020	2050	2080
Alps (n=12)	Dispersal	2.0 \pm 1.7	2.1 \pm 1.6	2.1 \pm 1.6	1.8 \pm 1.8	2.0 \pm 1.7	2.1 \pm 1.6	1.8 \pm 1.8	2.1 \pm 1.6	2.0 \pm 1.6
	No dispersal	6.6 \pm 0	6.6 \pm 0	6.6 \pm 0	6.6 \pm 0	6.6 \pm 0	6.6 \pm 0	6.6 \pm 0	6.6 \pm 0	6.6 \pm 0
Ardennes (n=1)	Dispersal	0	0	0	0	0	0	0	0	0
	No dispersal	0	0	0	0	0	0	0	0.5 \pm 0.8	0.8 \pm 0.9
Black Forest (n=1)	Dispersal	0	0	0	0	0	0	0	0	0
	No dispersal	0	0.5 \pm 0.8	0.5 \pm 0.8	0	0	0	0	0	0.5 \pm 0.8
Eifel (n=5)	Dispersal	0	0	0	0	0	0	0	0	0
	No dispersal	0	0	0	0	0	0	0	0	0
European Lowlands (n=9)	Dispersal	0	0.7 \pm 2.1	0.7 \pm 2.1	0	1.0 \pm 2.1	1.0 \pm 2.1	0	4.9 \pm 0	4.9 \pm 0
	No dispersal	0.5 \pm 1.6	6.2 \pm 2.8	6.2 \pm 2.8	0	4.9 \pm 0	4.9 \pm 0	0	4.9 \pm 0	4.9 \pm 0
Fennoscandia (n=5)	Dispersal	0	0	0	0	0	0	0	0	0
	No dispersal	0.3 \pm 0.7	0.3 \pm 0.7	0.3 \pm 0.7	0.3 \pm 0.7	0.3 \pm 0.7	0.3 \pm 0.7	0	0	0
North Jura (n=14)	Dispersal	0	0	0	0	0	0	0	0	5.7 \pm 4
	No dispersal	0	0	0	0	0	0	0	0	6.6 \pm 3.5
South Jura (n=3)	Dispersal	0	0	0	0	0	0	0	0	0
	No dispersal	0	0	0	0	0	0	0	0	0
Madeleine (n=0)	Dispersal	-	-	-	-	-	-	-	-	-
	No dispersal	-	-	-	-	-	-	-	-	-
Massif Central (n=8)	Dispersal	0	0	0	0	0	0.3 \pm 1	0	0	10.2 \pm 4.7
	No dispersal	0	0	9.2 \pm 1.4	0	0	10.2 \pm 1	0	0	12.1 \pm 1.6
Pyrenees (n=0)	Dispersal	-	-	-	-	-	-	-	-	-
	No dispersal	-	-	-	-	-	-	-	-	-
Vosges (n=0)	Dispersal	-	-	-	-	-	-	-	-	-
	No dispersal	-	-	-	-	-	-	-	-	-
Westerwald (n=3)	Dispersal	0	0.5 \pm 0	0.5 \pm 0	0	0	0	0	0	0
	No dispersal	2.5 \pm 2.6	4.9 \pm 0	4.9 \pm 0	0	0	0	0	0	0
Total loss of genetic uniqueness in Europe	Dispersal	2.0 \pm 1.7	3.3 \pm 5.2	3.3 \pm 5.2	1.8 \pm 1.8	3.0 \pm 3.4	3.4 \pm 3.3	1.8 \pm 1.8	7.0 \pm 1.6	27.4 \pm 6.3
	No dispersal	9.8 \pm 2.6	18.5 \pm 3	27.7 \pm 3	6.9 \pm 0.7	11.8 \pm 0.7	22.0 \pm 1.1	6.6 \pm 0	12.0 \pm 0.8	31.5 \pm 4.5

-: no unique allele enclosed in the mountain massif.

context of global change, it seems therefore interesting for the conservation of *L. helle* to focus on the populations with a high level of uniqueness and threatened with extinction. This is expected to contribute to ensuring the integrity of the entire intraspecific genetic diversity required to maximize the adaptation potential of the species and to preserve its evolutionary perspective in the future (Coleman et al. 2013; Pauls et al. 2013). Therefore, we provide an innovative approach that makes use of existing techniques to allow identifying the mountain systems where populations of *L. helle* with high levels of genetic uniqueness are predicted to be most threatened with extinction under global change.

We have shown that the predicted range shifts of *L. helle* and the subsequent population extinctions may be responsible for the loss of up to one third of the intraspecific genetic uniqueness found over Europe under the most severe global change scenario and dispersal assumption. A small number of regions in Europe (e.g. Alps, European Lowlands, North Jura and Massif Central) will likely contribute to an important part of the loss of genetic uniqueness. Although these regions are also those that currently have populations with the highest level of unique alleles, this pattern should not be taken as a rule of thumb and it may turn to be different for other species depending on their geographical distribution and large-scale genetic structure.

In *L. helle*, proactive conservation measures should, therefore, focus on these regions in order to maintain the intraspecific genetic diversity of the species in the future. In this respect, the populations from the Massif Central are of particular concern since our results suggest that they account for an important part of the genetic uniqueness in Europe and may disappear between 2050 and 2080. Interestingly, we have also found that the predicted loss of genetic uniqueness was not as marked as the population extinction across Europe. This indicates that at least some of the populations predicted to become extinct in the future enclose a small number of unique alleles or no unique allele at all (e.g. Pyrenees).

Our results also showed that the predicted extinction of the populations may be much lower if the species was assumed to be able to disperse at a reasonable distance in space and over time. Depending on the global change scenario, the loss of genetic uniqueness may, however, not be considerably reduced compared to the 'no dispersal' assumption. This indicates that a large part of populations with unique alleles are located in isolated and/or fragmented areas and will likely become extinct in the future because they will be unable to colonize newly suitable areas. These results draw attention to the importance of promoting the dispersal of *L. helle* in the future through the management of well-connected habitat networks (Barbet-Massin et al. 2011; Baguette et al. 2013; Stevens et al. 2013), especially in those regions where the loss of genetic uniqueness is expected to be high. Furthermore, this would preserve the continuous gene-flow among local populations required to prevent their genetic impoverishment and to maintain their high level of genetic diversity (Habel and Schmitt 2012).

When promoting spontaneous dispersal is not possible, the translocation of endangered populations including particular genetic diversity to other suitable areas has been recently advocated (Hoegh-Guldberg et al. 2008; Dawson et al. 2011; Thomas

2011). This assisted colonization is controversial and cannot be considered as the standard solution to preserve biodiversity at the global scale (Chapron and Samelius 2008; Davidson and Simkanin 2008; Huang 2008; Ricciardi and Simberloff 2009; Sandler 2010). Nevertheless, in some particular cases, as with species action plans for relict species, the relevance of assisted colonization to manage intraspecific genetic diversity should be scientifically assessed and may be carefully considered as a new proactive conservation strategy in response to environmental change (Carroll et al. 2009; Turlure et al. 2012).

It is worth to note that predicting the future distribution of a species is typically subject to uncertainties and limitations. Dealing with uncertainties is an important feature of biodiversity conservation practice. Here, we addressed some of the widely recognised limitations in species distribution modelling; we combined climate and land use change scenarios and we accounted for the limited dispersal ability of the species (Thuiller 2004; Araújo et al. 2005; Pearson et al. 2006; Guisan et al. 2006; Heikkinen et al. 2006). However, a refinement of the dispersal assumptions with the use of proxies that better reflect the dispersal capacities of the species in space and over time would provide more realistic predictions of future species distribution (Buse and Griebeler 2011; De Cáceres and Brotons 2012; Stevens et al. 2013).

It is also warranted to incorporate parameters related to genetic selection and species adaptation along with dispersal abilities in the modelling approach because local and phenological adaptations, genetic evolution and phenotypic plasticity are known to influence the persistence of species in response to global change (Cobben et al. 2011; Buckley et al. 2012; Arenas et al. 2012). There have been very few studies so far that integrated such processes into mechanistic species distribution modelling (Atkins and Travis 2010; Hamann and Aitken 2013; Kubisch et al. 2013). However, this type of approaches could be used to assess whether isolated populations are able to persist when dispersal is impossible.

Another important improvement to our methodological approach would be to incorporate information on functional (i.e. non-neutral) intraspecific genetic diversity for the species. There is an inherent link between the level of intraspecific genetic diversity and the adaptation potential of the species, but this link remains poorly understood in most of the species and is expected to be better specified with the emergence of new genome sequencing techniques (Angeloni et al. 2012; Pauls et al. 2013). Non-neutral genetic diversity is the only genetic basis for variation in life history traits which, in turn, determines whether the species is able to adapt to changing conditions (genetic polymorphism) (Fischer and Karl 2010; Schwander and Leimar 2011; Franke and Fischer 2013). Therefore, instead of blindly considering both neutral and non-neutral genetic diversity in the analyses, it would be more relevant to focus only on the latter. This would constitute a crucial step forward because focusing on non-neutral genetic diversity will likely allow refining conservation strategies to preserve the most functional part of the species adaptation potential under changing environmental conditions (Bonin et al. 2007). Nevertheless, the non-neutral part of the intraspecific genetic diversity remains

currently unknown for most of the species due to logistical reasons (Bonin et al. 2007; Pauls et al. 2013). Hence, there is now a growing interest to improve methods able to detect the functional part of the genetic diversity in order to better understand its effects on the adaptation potential of the species, in line with a number of studies on the genetic basis for dispersal behaviour in invertebrates (Wheat et al. 2010; Hanski 2011).

In this chapter we provided an innovative methodological framework that combines species distribution modelling techniques and large-scale genetic assessment to evaluate how global change may impact the genetic uniqueness of range-shifting species. This constitutes one of the most burning issues in conservation biogeography (Scoble and Lowe 2010; Bálint et al. 2011; Alsos et al. 2012; Funk et al. 2012; Coleman et al. 2013; Pauls et al. 2013).

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Results of two introductions of *Lycaena belle* in France

Henri Descimon^{1*} & Philippe Bachelard²

¹Laboratoire de Systématique évolutive, Université de Provence (case 5), F-13331 Marseille Cedex 03, France

²Société d'Histoire Naturelle Alcide d'Orbigny, F-63170 Aubière, France

*Corresponding author: Henri Descimon, Laboratoire de Systématique évolutive, Université de Provence (case 5), 3 Place Victor Hugo, F-13331 Marseille Cedex 03, France, E-Mail: Descimon.henri@free.fr

Abstract

The Violet Copper has been introduced in two medium or low elevation French mountain massifs, the Morvan and the Forez, where it was absent despite of the existence of suitable habitats. In the Marvon, six females from the French Ardennes were released in 1975; in the Forez, 15 females from the nearby Madeleine Mountains were used as colonists in 1992. In both cases, a core population was readily formed, and stepwise colonisation took place at a speed of about 500 m per year. Unfavourable zones of more than one kilometre were crossed, as were the watersheds between different hydrographical basins. This process generated a network of populations with a diameter of more than 10 km. While the Forez colony continues to thrive and to spread, the Morvan one has been struck by the general decline of the lepidopteran fauna in this region. Global warming but even more habitat destructions seem to be responsible of this degradation.

INTRODUCTION

Introducing a living organism in a place where it does not naturally occur is a debatable action. As for species originating from different biogeographical realms, things are clear: there are innumerable cases of ecological catastrophes due to the introduction of alien plants, animals or microorganisms in a region where their natural limiting factors are absent. Therefore, such kinds of introduction must be considered with utmost precaution – albeit many are still going on involuntarily, as, for instance, that of the “Palm Castnid” (*Paysandusia archon*), which is presently ruining the palms of the Mediterranean region and the associated landscapes. Not to forget that these palms are mostly issued from introductions!

From a conceptual point of view, when the transport of representatives of a species takes place within its natural distribution area at large scale, it should be better named “translocation”, a term which implies a less intrusive action, unlikely to exercise noticeable perturbations upon the extant natural equilibriums. Actually, often, this type of “introduction” could rather be a “reintroduction”, since the concerned species could well have been formerly present in the place of introduction. For instance, Demoly (2003) provided a convincing demonstration that many tree species introduced to Europe from extant Far East populations lived in our countries in a relatively recent past. This is especially true for species with a presently fragmented distribution area and, in particular, those usually characterised as “glacial relicts”, among which *L. helle* holds an eminent place (Finger et al. 2008, Varga and Schmitt 2008).

In such cases, the *pros* and *cons* on such introductions are rather ideological, ethic or even metaphysic: for instance, is it legitimate to modify (“pollute”) the fauna or flora of a region by adding new components to its ecosystems or even to force organisms to live in a place where they have no ancestors? Conversely, one might argue that introductions “enrich” the fauna or flora of a region and increases its biodiversity, or even “beautifies” its landscapes; less metaphysically, could founding new populations of a species improve its chances of survival through “assisted dispersal”? And, last not least, could this kind of “experiment” (or rather “faunal manipulation”, since the term of “experiment” seems immodest) generate fruitful scientific studies?

The case of *L. helle*, which we present here, seems to be a typically benign one. As it clearly appears elsewhere in this book, the large scale distribution of this butterfly covers a large area in western Europe, from Scandinavia to the eastern Pyrenees, but, at closer scale, it is strongly fragmented – a typical feature of the aforementioned “glacial relict” type (Finger et al. 2008, Varga and Schmitt 2008, Habel et al. 2010). A characteristic feature of the distribution of *L. helle* in France is that there are a lot of regions where it could be present, but actually is not (Bachelard and Descimon 1999). In these sites, the overall ecological and climatic conditions, the presence of the single food plant and of structures fulfilling its habitat prerequisites seem to call for its existence. However, careful and extensive prospections failed to disclose it, which allows to state that there are no established populations in these regions. A “suitability index” was contrived by HD (Bachelard and Descimon, 1999) – a more elaborated version of such a predictive parameter has been developed by Habel et al. (2010). Synthesising the factors favourable or unfavourable for the presence of *L. helle* proved to be able to predict its presence or its absence in a given region in most cases. However, a few of them were devoid of this species in spite of a favourable suitability index.

Among such “empty zones”, a small Hercynian massif of moderate elevation (902 m), the Morvan, appeared as a “missing link” between four regions where *L. helle* is present: the Madeleine mountains, the Jura, the Vosges and the Ardennes. It is located not very far from them (130 km, 210 km, 230 km, and 330 km, respectively) (see Fig.1). During two years in the sixties of the last century, HD resided in this region and was struck by the resemblance between the Morvan landscapes and those of these other low



Figure 1: Locations of introduced populations in the Morvan and Forez region. The site of introduction in 1975 in the Rainache Glen is marked.

western European mountain massifs – and by the absence of the Violet Copper. Quite naturally, the “ecological sibling” of *L. helle*, *Proclissiana eunomia*, was associated with it in this impression of “something lacking”.

This situation prompted HD to make what he called an attempt to “experimental biogeography” (Descimon 1976) by releasing females of *L. helle* and *P. eunomia* in the Morvan in the beginning of the seventies. Actually, it is the second species, which has focused most of the attention of HD and his co-workers and has given rise to some publications (Barascud and Descimon 1992, Nève et al. 1994, 1996, 2000, Barascud et al. 1999); the case of the Violet Copper has been briefly studied in faunistic papers (Descimon et al. 1980, Bachelard and Descimon 1999). Later on, a lepidopterist living in the Massif Central (who wishes to remain anonymous) performed another introduction in the Forez massif in 1992. The two authors of this chapter were informed of this introduction and followed the evolution of the founded colony.

The Morvan region was visited as regularly as possible by HD, while it is PB who surveyed the Forez one; from the 1990s onwards, HD gave up with this study, but went back to the Morvan in 2011 and 2012, while PB could reach more easily and regularly his study zone. We expose here the results of these observations, which cover almost forty years for the Morvan and twenty for the Forez.

THE MORVAN INTRODUCTION

The Morvan massif is located at the south-eastern border of the Paris basin at the watershed between the Seine drainage, which flows into the Channel, and the Saône drainage, which leads into the Mediterranean. Cut by the 47th parallel and covering grossly 60 km from the North to the South and 40 km from the East to the West, it is a crystal-line Hercynian massif included in a sedimentary region; its elevation is moderate and increases gently from its northern part, where it does not exceed 500 m, to its southern one, which reaches 902 m at the Haut Folin summit. There is also a marked contrast between the morphologies of these two parts: the northern one consists of gently rolling hills with rather large, peaty and boggy treeless basins, where *Boloria aquilonaris* and *Coenonympha tullia* used to thrive until the 1960-1970 years, prior to the first effects of global change. One must underline that the last colonies of these species have vanished during the end of the 1990s, including specifically managed areas.

Polygonum bistorta, the food plant of *L. helle*, scantily grows in these large open areas and is limited to their borders, to the marshy banks of rivers or to neglected corners of meadows, where the impact of cattle grazing is not too high. The southern part of the Morvan is more mountain-like, with deep glens and steep slopes; peat bogs are small and located at the head of river basins. Most of the slopes and ridges are covered by deciduous forests where Beech prevails (and, in particular, the Anost forest). During the 1960s and 1970s, some sheltered clearings harboured dense colonies of *P. bistorta* in the thalwegs obviously favourable to the Violet Copper. These clearings were generally issued from formerly grazed meadows, abandoned because of their isolation; the proliferation of *P. bistorta* was a transient phenomenon, which preceded the colonisation by Chestnuts and, finally, Beech forest.

Among these potential habitats, a valley located upstream to the village of Lavault de Frétoy (Nièvre department) in southern Morvan, the Rainache glen, looked especially favourable to the two *P. bistorta* hosts, *L. helle* and *P. eunomia*. Actually, the first introduction was performed in 1970 and, only with four females of the second species, at Saint Brisson in northern Morvan; the Rainache glen had not been spotted and the more favourable character of southern Morvan was not acknowledged. Mainly for practical reasons, the small colony of *L. helle* living in the French part of the Ardennes massif, at Pont Colin (Ardennes department), close to the frontier between France and Belgium, was chosen as a source population for the introduction experiment (the same choice was made for *P. eunomia*). Furthermore, it was easy to make a there and back

journey from Paris to the Ardennes and another one to the Morvan on the following day, the butterflies being kept fresh in an ice chest. However, an inconvenience of this translocation was the small size of the *L. helle* population living in the Ardennes, not allowing to sample many individuals.

The first release of *L. helle* was performed with only two females in a branch of the Rainache brook at 650 m elevation on June 12th 1973; by the same time, 14 females of *P. eunomia* were released a little below the former site along the main stream. In both cases, *P. bistorta* formed a dense colony, mixed with *Ranunculus aconitifolius* and *Caltha palustris*, whose nectar is greedily sipped by *L. helle*. After their release, the females of both species became active and started laying eggs on their food plants.

One year later, on June 4th 1974, rather numerous *P. eunomia* males were observed on the release site and 200 m below and above; transect counting indicated that around 100 individuals were on the wing, with only a few females, which is unsurprising owing to proterandry. On the contrary, careful inspection of the place where the two *L. helle* females were released did not allow to spot a single individual of this species. This trial is likely to have failed.

On June 7th 1975, six females of *L. helle* from Pont Colin were released in the Rainache valley. In the following year, around ten individuals could be observed while, in 1977, a population composed of a considerable number of individuals occupied all the favourable zones of the Rainache valley over 2 km upstream and downstream to the release site. This implies that colonist females were able to cross forested zones of at least 200–300 metres. In the end of the 1970s, a large population of several hundreds of individuals, actually more numerous than the original population of the French Ardennes, lived in the terminal basin of the Rainache valley, a place once held by the “Maquis Socrate” during World War II. The colonization took place also downstream, below the small village of Lavault de Frétoy until a series of marshy meadows at Fou de Verdun, 4 km from the release site and at an elevation of 450 m. This also implied crossing an inhabited zone, fields and grazed meadows. This colonizing power was, however, limited to the Lavault de Frétoy hydrographical basin and, at that time, *L. helle* had not crossed the watershed between this basin and the neighbouring ones.

During the 1980s, the populations of *L. helle* and *P. eunomia* could not be surveyed. In the following decade, studies about population genetics and dynamics were undertaken using the latter species. Using this opportunity, data on *L. helle* were casually gathered (Bachelard and Descimon, 1999). On May 30th 1991, a sample was collected at Lavault de Frétoy (Maquis Socrate) for further use in allozyme and microsatellite DNA analyses (Habel et al. 2010).

In June 1994, a colony of the Violet Copper was observed by B. Barascud at L'Huis Prunelle in a cluster of wet neglected meadows belonging to the Cure river basin; this habitat is located on a plateau at an elevation of between 650 and 700 m. Although this site does not lie in a sheltered glen as the Rainache valley, high edges and forest skirts provide balmy habitats suitable for the Lycaenid. This location represented a modest step forward, 5 km away from the thriving colony of Maquis Socrate; however, it is clear

that the dispersing females must have crossed rather large continuous stretches of Beech and Conifer forest of over 3 km.

In the following year (1995), C. Dutreix discovered a restricted population of *L. belle* in the upper Canche valley, at about 600 m elevation, in the heart of the Haut Folin massif; worth noting is that HD and B. Barascud explored this locality in the previous year without observing the butterfly. Its arrival was thus likely to be very recent; in 1996, B. Barascud, C. Dutreix and HD observed that the population had markedly increased. In the same year, HD discovered another population of *L. belle* at the north-eastern border of the Folin massif, at the head of a rill flowing towards the hamlet of Le Pommoy (650 m); there, *L. belle* flew jointly with *P. eunomia*, which had not yet reached the Canche valley (what it did later on). The habitats available to *P. bistorta* hosts in the Folin massif are scanty; indeed, this area is densely forested, the streams are torrent-like and the general relief is steep – the invasion of this region therefore is the most striking. More anecdotally, doing so, *L. belle* has invaded the drainage basin of the Mediterranean sea. The *L. belle* colony present in the Morvan at that time covered an area of more than 10 km in diameter.

From the end of the 1990s onwards, no more survey was performed in the Morvan; three individuals (presently conserved in the Natural History Museum of Autun) were collected by D. Morel on May 27th 2000 in the lower Canche valley (400–500 m), close to the village of Roussillon en Morvan. *L. belle* was observed at Ruisseau Morin (650 m), ca. 1 km WNW of the already known locality of L'Huis Prunelle on April 28th 2010 by A. Ruffoni (pers. comm.).

At the opportunity of the edition of this book, and in the perspective of completing the present work, HD and his wife (AD) planned a trip to the Morvan in 2011 to monitor the status of *L. belle* in recent times. It was decided to cross the Morvan from the South to the North. The first place to be explored, on May 29th, was the Canche valley and the neighbourhood of Roussillon en Morvan. In spite of careful inspection, no Violet Copper could be observed. The general density of butterflies was stunningly low, at an epoch in which butterflies used to thrive in the past. The vegetation looked burnt by drought and excessive heat, and the butterfly flight period advanced by almost one month – a fact confirmed by local people and entomologist colleagues (R. Essayan, D. Morel and A. Ruffoni).

The following targets were obviously the plateau of L'Huis Prunelle and the Rainache valley. There, another bad surprise expected us: although forestry has practised harsh methods for a long time in the Morvan, the advent of timber industrial management has deeply changed the landscapes and the habitats of this region. In particular, the marshy meadows had been drained and planted with Douglas fir, letting no more space for *P. bistorta* and its hosts. In the zones not (yet) struck by intensive forestry, abandonment allowed the already mentioned closure of the remnant clearings. It was thus impossible to HD and AD to find the habitats precisely spotted within the Anost forest and the Rainache valley during the 1970s.

On the plateau of L'Huis Prunelle, in an abandoned marshy meadow, one single very old male of *L. belle* was glimpsed. On the contrary, farther North, extensive

prospection of all the numerous apparently favourable spots, which surrounded the region of the Settons lake, did not allow to observe any Violet Copper individual as did, still further, the region of Saint Brisson. A long period of bad weather precluded continuing the research of other sites where *L. helle* could be present; moreover, it was clear that, on this precocious year, the flight period of the butterfly was practically finished.

Two important remarks must be put forward: first, there is absolutely no record of *L. helle* north to L'Huis Prunelle and Ruisseau Morin all along the 37 or 39 years after the introduction of this species; therefore, there is no indication that it has ever colonized the northern part of the Morvan. Second, on the contrary, *P. eunomia* has completely invaded all the available habitats of the massif as soon as the 1990s (Barascud et al. 1999). In 2011, it was present everywhere, being far the most abundant of the Fritillaries and even of the butterfly species.

Since we considered that the results of the exploration carried out in 2011 were insufficient, the Morvan was visited again in 2012. Potential sites, which could shelter colonies of *L. helle*, were carefully researched using large scale maps and GPS. Two trips were accomplished on May 13th and 24-26th. They allowed a thorough coverage of the places where the presence of the Lycaenid could be purported, including places accessible only through long walks, although most of them have vanished in recent years, as mentioned before. However, in spite of a very warm, sunny weather, the general density of butterflies was still lower than in the previous year and no *L. helle* was observed all along the excursions. Obviously, the butterfly flight periods were as delayed as they were early in the previous year. In particular, *P. eunomia* was not yet on the wing – only one very young male of this species was observed during the last day of the trip. In the past, this should have been auspicious, since the Lycaenid's flight period precedes the Nymphalid's one by two or three weeks; at present time, one must redoubt that the Violet Copper has no more flight period at all in the Morvan. R. Essayan, A. Ruffoni and D. Morel kindly looked for *L. helle* in the most significant localities such as L'Huis Prunelle in the beginning of June, and still in vain.

THE FOREZ INTRODUCTION

The Forez mountain ridge stretches between the 45th and 46th parallels, in the heart of the French Massif Central, over 40 km from the NNW to the SSW; its width is around 10 km, between the Allier and Loire river basins. It reaches rather high elevations – 1640 m at Pierre-sur-Haute – and presents a vigorous topography, with steep slopes and few, if any, peaty tablelands. Still, many valleys present marshy meadows full of *P. bistorta*, apparently quite favourable for *L. helle*. However, in spite of intensive research, neither HD nor PB did discover this species all along their prospections (Bachelard and Descimon, 1999); the absence of the Violet Copper was confirmed by the local lepidopterists Roland Bérard and Claude Colomb (pers. comm.). Two points rendered

this absence paradoxical: (i) the suitability index of the Forez was clearly higher than the threshold, which allows predicting the presence of *L. belle*; and (ii) the Madeleine mountains, which look like a northern extension of the Forez, possesses colonies of the *Lycaenid*. The distance between the two massifs is rather short (around 30 km) and the intervening region does not lie very much below 500 m; it is surprising that colonist butterflies should not have overcome these moderate obstacles.

On May 30th 1992, 15 females and 3 males of *L. belle* originating from the Madeleine Mountains were released in the southern Forez mountains, on the territory of the commune of Saint Anthème (Puy-de-Dôme department), at an elevation of 1150 m (Bachelard and Descimon 1999). On May 16th and 31th of the following year, five imagoes were observed; in further years, the size of the population present on the release site increased steadily (Table 1). In May and June 1996, over 80 individuals were observed in this site and around 40 at a distance of 300 to 500 m from it. Since this time, expansion is going on. This progression has generally taken place following the watercourses and both downstream and upstream; however, as in the Morvan, strips of unfavourable habitat (forest or dry grassland) must have been cleared. In 2012, the limits of the colonised area lie 2.5 km from the release point towards WSW and 9 km towards NNE. Interestingly, the watershed between the Allier and Loire basins has been jumped over prior to 2009

Table 1: Introduction of *Lycaena belle* in the Forez; Location, number of observed individuals, distance and orientation from original release place.

D	M	Y	Commune	Place	Observer	Number	Km	Direction
16	5	1993	Saint-Anthème	Barlot – le Chomet	C. Colomb	1		
31	5	1993	Saint-Anthème	Barlot – le Chomet	C. Colomb	4		
5	6	1994	Saint-Anthème	Barlot – le Chomet	C. Colomb	9		
21	5	1995	Saint-Anthème	Barlot – le Chomet	C. Colomb	7		
28	5	1995	Saint-Anthème	Barlot – le Chomet	C. Colomb	30		
11	6	1995	Saint-Anthème	Barlot – le Chomet	C. Colomb	4		
9	6	1996	Saint-Anthème	Barlot – le Chomet	C. Colomb	120		
	6	2001	Saint-Anthème	2km downstream from release	C. Colomb	Not counted	2	S
		2001		Barrage des Pradeaux	C. Colomb	D°	2,5	WSW
		2003	Saint-Anthème	Ruisseau Grand Genêt- Pont	C. Colomb	D°	4,5	N
15	6	2004	Saint-Anthème	Ruisseau Grand Genêt	P. Bachelard	2	6	N
20	6	2006	Saint-Anthème	Ruisseau Grand Genêt	P. Bachelard	2	6	N
20	6	2006	Valcivière	Ruisseau des Pradeaux	P. Bachelard	1	3	WNW
16	6	2009	Saint-Anthème	Ruisseau Grand Genêt	P. Bachelard	5	6	N
24	6	2010	Roche	Grand Pierre Bazanne	P. Bachelard	5	7	NNE
8	6	2012	Roche	Grand Pierre Bazanne	P. Bachelard	1	7	NNE
8	6	2012	Saint-Anthème	Les Fumées	P. Bachelard	1	4	NNE
15	6	2012	Roche	Probois	P. Bachelard	18	9	NNE
15	6	2012	Roche	Tourbières de Gourgon	P. Bachelard	1	8,5	N

and the Violet Copper has pursued downstream its conquest. In the southern part of the Forez, this butterfly has become one more constituent of the butterfly fauna.

DISCUSSION

First and in the light of the ideological question of the opportunity and legitimacy of the introductions of the Violet Copper, it looks clear that the two concerned translocations did not give rise to an excessive proliferation of this species. Actually, it seemed perfectly at home in the new habitats, which were rendered available to it and might have persisted in these areas since the end of the last ice age. These impressions, of course, are subjective. Furthermore, to the rather moot question: "have the translocations of *L. helle* been detrimental to the Morvan and Forez ecosystems?" it is not too risky to answer "no".

To the reciprocal question: "have these translocations been detrimental to *L. helle*?" the answer is still more obviously "no". The cost of these faunal manipulations is limited to eight (two + six) females for the Morvan introduction and to 15 females for the Forez one. Now, the original populations of the French Ardennes and of the Madeleine mountains are both endangered; if the colony of Pont Colin is actually an outlier of the large (but declining) populations of the Belgian Ardennes, the Madeleine one, both isolated and occupying a restricted area, ranks among the most critically endangered of this endangered species (Bachelard and Descimon 1999, Van Sway and Warren 1999). Increasing the size of these populations and dispersing them in empty but favourable regions could improve their chances of survival. One might even integrate such actions in global management measures; they are less costly than habitat restoration, to which, of course, they cannot be substituted. Actually, the problem which is then raised is the level of human intervention, which is advisable and tolerable to rescue living species and their gene pools; this topic is well beyond the scope of the present contribution.

These faunal manipulations have brought a bit of new information about the biology of *L. helle* and especially upon its dispersal abilities; this species is reputedly quite sedentary and unable to migrate over distances larger than a few hundreds of metres (Fischer et al. 1999). Now, our experiments have shown that colonist females are able to cover distances superior to one kilometre crossing unfavourable habitats as dense, continuous forest. In the Morvan, a distance of 11.5 km (between the Rainache initial release and the Canche valley) has been bridged in 20 years (1975-1995); over a similar time period, but later (1992-2012), it is a distance of 9 km which has been covered in the Forez (Fig. 1); one may therefore coarsely estimate the average progression to 0.5 km per year. According to our observations, this progression is likely to have been stepwise, giving rise to an "oil spot"-like metapopulation. The features of the invasion of the Morvan by the other Bistort host, *P. eunomia*, are rather similar (Nève et al. 1996). In this species, the foundation of the colony and its further dispersal have been accompanied by (predictable) changes in the genetic structure of its populations (Barascud et al. 1999); although this point has not been specifically addressed in *L. helle*, it seems that noticeable changes in

genetic structure have taken place between the mother population of Pont Colin and its descent, which invaded the Morvan (Habel et al. 2010c).

As clearly shown by Turlure et al. (2009), *L. helle* and *P. eunomia* are ecological “false twins”. While the second has invaded all the suitable habitats of the Morvan, the former is still confined to the southern part of this massif. This does not mean that it was slower or less dynamic; indeed, *L. helle* preceded *P. eunomia* in the Haut Folin region, at the southernmost limit of the zone presenting habitats suitable for both species. One could claim that *P. eunomia* has been first introduced in northern Morvan in 1970 and then in southern Morvan in 1973, i.e. before *L. helle* (Descimon 1976), but it is hard to believe that this fact alone might explain the confinement of the Violet Copper to the southern part of the massif. It is much more likely that *P. eunomia* is less exacting and is able to live in perturbed habitats, at a relatively low elevation (Turlure et al. 2009), conditions which prevail in the northern Morvan. Often, in the regions where *L. helle* is naturally present, one can observe, not far from localities where it thrives, apparently suitable habitats which are not occupied (Descimon 1964, 1965, Bachelard and Descimon 1999), and this simply because they are too low, with the frontier often being sharp. This is likely to be the case also in the northern Morvan, where, in spite of the presence of many apparently suitable habitats, elevation is insufficient everywhere. In the southern part of the massif, the, slightly, higher elevation and more mountain-like topography could have provided conditions more favourable, at least for a while.

If this is true, it could well explain the recent decline (and perhaps extinction) of the populations of *L. helle*, observed in 2011 and 2012 in the southern Morvan. These events could represent a rehearsal of the scenario presented by Habel et al. (2010): the limited “altitudinal reserve” of the southern Morvan has not allowed the biota present there to withstand one of the first climatic shocks, which struck Western Europe during recent years. According to R. Essayan, the Morvan has lost 13 species of butterflies during the last 20 years. Furthermore, the confrontation of HD’s 1970s memories with the present situation is staggering. Actually, sampling or studying any butterfly species, even the very common ones, should have been a challenge in 2011 and 2012. Could the observed situation be accidental and could more “normal” conditions come back? Previous observations made on the extinction of low-elevation colonies of *Parnassius apollo* in SE France suggest that extinction occurs as a consequence of extreme climatic events, notwithstanding the fact that the population could have thrived immediately before (Descimon 1994). This fact should be kept in account in the extinction scenarios linked to climate change (Habel et al. 2010 a,b).

Things seem to be less dramatic in the Forez, where *L. helle* has not displayed the least sign of decline and has even extended its area in recent years. It is likely that it will colonise the entire massif in the near future. The altitudinal reserve in these mountains is of the same order as that of the Sancy massif – 400 to 500 metres. Facing the relentless rise of climatic zones, the populations of the Violet Copper introduced in the Forez is granted a precarious reprieve. However, one must not forget that there were reasons to the fact that it was naturally absent in the Forez, maybe continental or even Mediterranean influences.

CONCLUSION

The introduction of *L. belle* in the Morvan and the Forez have provided some insight into its ecology, population dynamics and conservation. The rather fast colonisation of the two massifs shows that an apparently sedentary species is able to perform efficient dispersal and even to overcome rather large unfavourable spaces. Translocation of endangered populations with a unique genetic structure could be integrated in a global management scheme. At present, the fates of the two colonies seem to be different. In the Forez, *L. belle* is building a rather prosperous and apparently stable (meta)population. But for how much time? In the Morvan, after a promising start, it has been limited in its extension by conditions, which are at the borderline of suitability, especially because of an insufficient elevation, and perhaps still more by the destruction of its habitats; and the recent vagaries of the climate could well have inflicted it the finishing stroke. Anyway, if global warming (and larger stochasticity) of the climate is still going on, it is not only *L. belle*, but practically all butterfly fauna which will collapse, as it is already the case in the Morvan.

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Restoration and management of habitat networks for *Lycaena helle* in Belgium

Philippe Goffart^{1*}, Eric Cavelier², Patrick Lighezzolo²,
Alexander Rauw² & Dominique Lafontaine²

¹Département d'Etude du Milieu Naturel et Agricole (DEMNA), Service Public de Wallonie (SPW), B-5030 Gembloux, Belgium

²Natagora, B-5000 Namur, Belgium

*Corresponding author: ¹Département d'Etude du Milieu Naturel et Agricole (DEMNA), Service Public de Wallonie (SPW), Avenue Maréchal Juin, 23, B-5030 Gembloux, Belgium, E-Mail: Philippe.Goffart@spw.wallonie.be

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Abstract

To cope with the fragmentation of natural habitats in Western Europe, one of the main causes of biodiversity decline, restoring habitat networks suitable for threatened species is an urgent need. A Life+ project has been launched in 2009 in Southern Belgium aiming to restore habitats at the landscape-scale for three butterflies protected in the European Union, including the Violet Copper, *Lycaena helle*. After a brief factual introduction about the project and its extent, we outline the Belgian status of *L. helle*, the habitats and resources it uses, and also the threats to its populations. Then, we try to define the necessary constitutions of a self-sufficient habitat network for this butterfly, in terms of size of suitable habitat patches and distances between them, based on available information in the scientific literature. Afterwards, we review and describe the methods of habitat restoration, re-creation and management that seem most appropriate and which are currently applied and tested in our Belgian Life project. We conclude with some first global results and future prospects of the project, and finally identify the remaining gaps in the knowledge and relevant issues that deserve further research for more effective actions. Cost ranges are given in an appendix for the information of managers.

INTRODUCTION

Habitat loss and fragmentation due to human land use are considered primary drivers of species decline and extinction on earth (Brook *et al.* 2008), through reductions of population sizes and increases of their isolation (Hanski 1999). The idea to restore “ecological networks” to promote survival of populations in fragmented landscapes hence made its way, in connection with the emergence of the concept and theory of metapopulations, since the beginning of the 1990s (Hanski 1997; Jongman and Pungetti 2004). The “linkage strategy” that aims to create stepping stones or corridors binding local habitat patches offers an attractive method for its practical application in conservation planning (Baguette *et al.* 2013).

For butterflies, which suffer under strong decline over Western Europe (Van Swaay and Warren 1999; Maes and Van Dijk 2001; Konvicka *et al.* 2006), the restoration of habitat networks appears highly desirable, as they exist predominately in metapopulation structures (Thomas and Hanski 1997). However, the implementation of landscape-scale restoration projects focused on these insects are still very few (but see Porter and Ellis 2011; Ellis *et al.* 2012). Yet, the European Life+ programs offer exceptional financial opportunities to restore habitat networks for Natura 2000 species of invertebrates, including butterflies (European Commission 2012), which could be further exploited in many regions over Europe.

This chapter aims to describe the measures taken in three different regions of Southern Belgium to restore and manage habitat networks for the Violet Copper *Lycaena helle*, a postglacial relict butterfly, under a (so-called) “Butterfly” Life+ project launched in 2009. Some researchers have opportunely stressed the need of evidence-based conservation practice to ensure effectiveness of interventions (Sutherland *et al.* 2004). Earlier conservation efforts on some threatened butterflies suggested, indeed, the importance to have a good knowledge of species autecology (Thomas 1991). A recent report about the successful recovery of the Large Blue butterfly *Maculinea arion* in England, after population collapse and final extinction in 1979 and subsequent reestablishment from Swedish populations, provided a definitive demonstration of this need (Thomas *et al.* 2009). Therefore, our project was designed by trying to rely as much as possible on available scientific literature about the requirements of the focused species (*L. helle*) in terms of habitats quality and spatial distribution, but also about the potential methods of restoration, re-creation and management of its habitats. This background information and the options chosen in this ongoing Life+ project are reviewed and outlined synthetically here, after brief presentations of the overall project framework and of *L. helle* status and threats in Belgium. The chapter concludes succinctly with first restoration results and future prospects.

THE “BUTTERFLY” LIFE PROJECT

A co-funded Life+ project has been introduced in 2007 and launched in January 2009 with the objective of restoration of metapopulations of the three Natura 2000 butterflies (Annexe II) present in Southern Belgium, including the Violet Copper, *L. helle* (beside

the Large Copper, *Lycaena dispar* and the March Fritillary, *Euphydryas aurinia*) (LIFE07 NAT/B/000039)¹. The project is supported by two partners: Natagora, the main Nature Conservation NGO in Wallonia, and the administration of the Walloon region. It was planned for five years but has been extended for one more year, until December 2014. The budget amounts to over 7 million Euros, including the commitment of nine persons. The project focuses on five areas, for a total of 25 Natura 2000 sites in the Ardenne's natural region and its surroundings. They were selected to maximize profits for the three target species, while working in areas not covered by previous Life projects. Three areas (16 sites) are inhabited by *L. helle* populations. The objective of the project is to restore and manage at least 250 ha in all for this species (70 ha in Northern Ardenne, 120 ha in Eastern Ardenne and 60 ha in Lotharingy).

STATUS, HABITATS AND THREATS OF *LYCAENA HELLE* IN BELGIUM

The Violet Copper is distributed across nearly all the Ardenne's massif, in connection with the Eifel massif in Luxemburg and Germany and runs over to the South in the northern part of Belgian Lotharingy, in the Semois high valley (Fichefet *et al.* 2008). The number of occupied habitat patches recorded during the last decade amounts to a few hundred, ranging from 250 to 650 meters above sea level. The species seems in decline in some areas, especially at low altitude along the northern boundary of the Ardenne's massif and along the southern margin in Lotharingy (unpublished data). Consequently, its conservation status is considered 'Vulnerable' in Wallonia (Fichefet *et al.* 2008), where it is legally protected since 2001.

In Belgium, *L. helle* is found mainly in abandoned humid grasslands dominated by the larval food-plant *Polygonum bistorta*, scattered with trees and shrubs or surrounded by forest edges (Fichefet *et al.* 2008). It also occurs along the forested margins of acid bogs, which were probably its primary habitat. It is absent from regularly mown meadows or heavily grazed grasslands and from open landscapes devoid of hedges, tree copses or forest edges.

The adult butterfly appears once a year, from April to July, and winter is spent as a pupa. Caterpillars are to be found under the leaves of the larval food plant from June until August. This abundant plant is the caterpillar's exclusive food plant in Belgium, like in all its Western European distribution, while other host plants (*P. viviparum*, *P. amphibium*, *Rumex acetosa*, *R. acetosella*, *R. aquaticus*) are used in Northern and Eastern Europe (Henriksen and Kreutzell 1982; Tshikolovets 2011). However, ecological studies on *L. helle* habitat requirements showed that the presence of large food plant patches is of course a necessary condition, but alone clearly is not sufficient (Goffart and Waeyenbergh 1994; Turlure *et al.* 2009, see Chapter IV, Turlure *et al.*), a finding that proved valid for many species of butterflies and justifies a functional resource-based approach for habitat definition (Dennis *et al.* 2003, 2006; Dennis 2010). Indeed, other ecological

¹ <http://www.life-papillons.eu>

resources (consumables or utilities) are used in the Violet Copper environment, such as a large variety of nectar flowers (herbs, shrubs or trees) visited by adults, sunny edges where males adopt a territorial perching strategy waiting for potential mates, tops of tallest trees selected as roosting sites during the night and under adverse conditions (Goffart and Waeyenbergh 1994; Goffart *et al.* 1995; Goffart *et al.* 2001; Turlure *et al.* 2009, see Chapter IV, Turlure *et al.*). Consequently, *L. helle*'s optimal habitat is formed by a mosaic of herbaceous and ligneous vegetations, at the interface of open areas and woodlands (Nunner 2006), which has fundamentally a transitional character (ecological succession), raising the question of its management (Fischer *et al.* 1999).

The main causes of the slight decline of the Violet Copper in Southern Belgium during the last two decades are:

- afforestation of open habitat patches by spruce (*Picea abies*) or hybrid poplar (*Populus x canadensis*);
- eutrophication of alluvial soils (Fischer *et al.* 1999; Bauerfeind *et al.* 2009), due to river pollution (villages, crops, intensive meadows upstream);
- conversion of abandoned meadows into intensive pastures;
- fertilization of meadows coupled with mowing, which leads to reduce *P. bistorta* dominance in grassland vegetation (Pechackova *et al.* 2010);
- the abandonment of humid grasslands since at least the mid-20th century, which has likely resulted in an increase of favourable habitats for *L. helle* at first, but then in a decrease of these habitats in recent decades, due to their 'closure' corresponding to the end of the succession process;
- rise of boar populations in the last decades and hence increase of their damages to alluvial vegetations;
- global warming, as simulations suggested that this species might disappear from Belgium before the end of the 21th century with predicted increasing temperatures (Maes *et al.* 2010; Habel *et al.* 2011).

All these decline drivers remain potential threats for the populations of *L. helle* in the future in Southern Belgium, despite the implementation of the new Natura 2000 legislation.

REINFORCEMENT OF HABITAT NETWORKS

The importance of habitat networks for butterfly (meta)population survival has now been recognized since at least two decades (Thomas and Hanski 1997). Besides the persistence of suitable habitat patches, their spatial arrangement and connectivity, with sufficiently close distances between them to allow for dispersal and thus for exchanges and colonisations of empty patches, appeared crucial for the conservation of specialised species (Schtickzelle *et al.* 2005; Bulman *et al.* 2007). Studies carried out in Western Germany (Fischer *et al.* 1999; Bauerfeind *et al.* 2009) showed that this was also the case

for *L. belle* and confirmed that habitat quality alone was insufficient to ensure population survival and that connectivity and patch size were essential factors. Indeed, a statistical model revealed that patch occupancy was predominantly affected by patch isolation and size and by the ground coverage of *P. bistorta* (Bauerfeind *et al.* 2009).

Therefore, a critical point in this project was to define the characteristics of a self-sufficient habitat network for the long-term survival of *L. belle* populations, and in more detail:

- Which number of interconnected habitat patches is necessary?
- What minimal size must have each habitat patch?
- At which maximal distance can two habitat patches be still considered as interconnected?
- What size must the whole interconnected network reach?

Published scientific literature can help answering some of these questions. In particular, comparisons of occupied and unoccupied patches in the Westerwald habitat networks in Germany give some useful insights (Fischer *et al.* 1999; Bauerfeind *et al.* 2009). The average distance between next occupied patches was 598 ± 405 m (median = 400 m) in the 1994 survey, which was significantly shorter than the distance between extinct populations to the next still existing population (1533 ± 1087 , median = 1250 m, $n = 12$). In this study, the mean size of an occupied patch was 1.98 ± 2.9 ha (median = 1 ha), the smallest having 0.2 ha in extent and the largest 20 ha. During a survey in the same area in the year 2004, performed with a much more detailed mapping of habitats, the mean distance of occupied patches to the next occupied patch was 261 ± 26 m ($n = 128$), while it was 641 ± 68 m for non-occupied patches ($n = 102$) and the average distance to the next three occupied patches was 520 ± 37 m (vs 891 ± 66 m for non-occupied patches). The average occupied patch size was also smaller: 0.75 ± 0.15 ha (vs 0.32 ± 0.06 ha for non-occupied patches).

These results can be explained by the apparent sedentary character of this butterfly, as all mark-release-recapture (MRR) experiments conducted on adults *L. belle* suggested a low dispersal ability and/or propensity, with average distances ranging from 37 to 108 m, according to sex and study, and maximal distances not exceeding 335 m for males and 522 m for females (Fischer *et al.* 1999; Chulunbaatar *et al.* 2009; see chapter 4, Turlure *et al.*). However, MRR experiments always underestimate total mobility and results 'may be related more to the size of the study area than to the species' actual dispersal ability' (Schneider *et al.* 2003). Indeed, recent intensive and large scale studies on butterflies revealed that long distances dispersal even exists in species that were long regarded as sedentary (Zimmermann *et al.* 2011). In the case of *L. belle*, some observed colonisation events and genetic analyses suggest that adults can actually move larger distances than estimated by MRR (Bachelard and Descimon 1999; Finger *et al.* 2009; see chapter IV, Turlure *et al.*). Interestingly, linear structures like forest edges seems to be favoured during flight movements and woods does not appear to represent strong barriers for adults according to the numerous exchanges recorded between clearings separated by sparse woodland belts (Fischer *et al.* 1999).

However, even if we have an estimate of the distances acceptable between habitat patches, we do not know precisely how much habitat network is needed for supporting a long-term viable population as no population viability analysis (PVA) or minimum viable metapopulation (MVM) simulation studies have been carried out on *L. helle*, like those that, for example, were applied on *Euphydryas aurinia* (Schtickzelle *et al.* 2005; Bulman *et al.* 2007). However, concepts and predictions derived from metapopulation theory, like minimum viable metapopulation (MVM) and minimum amount of suitable habitat (MASH), as well as empirical studies on butterfly metapopulations have highlighted that networks of at least 15 to 20 well-connected patches are required for long-term persistence of species with high occupancy rates of the patches; these figures can be retained as a rule of thumb (Hanski *et al.* 1996; Hanski 1997; Thomas and Hanski 1997).

Finally, from all this scattered and partial knowledge, we assumed conservatively that an adequate network for *L. helle* should have a minimum of 15 interconnected patch units of at least 0.3 ha in size, with a minimum of three patches > 1 ha, covering altogether an area > 10 ha, spatially arranged along rivers (considering valleys oriented dispersal) with inter-patch distances (to the next closer unit) not exceeding 1000 metres, preferably inferior to 500 m.

As a first step in the Life+ project, maps of extant *L. helle* populations and suitable habitats were drawn for each Natura 2000 site concerned. Potentially restorable sites were also mapped to construct operational plans, prioritizing restoration actions in valleys with too loose habitat network, with the above standards in mind. These plans are currently implemented as far as possible through ongoing habitat restoration and re-creation (Fig. 1).

HABITAT RESTORATION

This section focuses on the restoration of degraded humid grasslands. The degradation can have two main forms:

- Invasion by *Filipendula ulmaria* (or *Urtica dioica*) and / or by scrubs and trees (wil-
lows, birch, alder, aspen) due to abandonment of management and / or eutrophica-
tion (Schrautzer *et al.* 1996; Prévosto *et al.* 2011);
- Shortening of the vegetation and decrease of *P. bistorta* due to overgrazing (by cows,
horses or sheep) or game trampling or rooting (boar).

Summer mowing with hay removal can reduce dominance by *Filipendula*, frequent in many abandoned wet grasslands, and leave room to other plants including *P. bistorta* where it is still present (Goffart *et al.* 2001), but successive (annual) intervention may be required to get significant results (Delescaille 2000). Mulching is also an alternative, but is likely to give poorer results than annual hay making as there is no removal of nutrients (Bakker 2013). However, this method or, even better, milling can be convenient as a first restoration measure in rank vegetation, before moving on to mowing the next season, so as to obtain a sufficiently levelled ground, practicable with a cutter deck. In any case, low lift machines must be selected to avoid rutting and soil compaction, which can foster



Figure 1. *L. helle* habitat network restoration in the Our basin in the Belgian Eastern Ardenne. Legend: Natura 2000 site perimeter (dark grey line), rivers (light grey line), extant habitat patches still occupied by *L. helle* (black), recently restored plots (dark grey), potential patches for future restoration (light grey).

encroachment by soft rushes (*Juncus effusus*) (see Photo 1). However, the restoration may be illusory if strongly polluted rivers cross alluvial grasslands (eutrophication).

Control and partial removal of willows (*Salix* spp., including the most abundant *S. aurita* in the Ardenne's massif) and other trees (*Betula*, *Populus tremula*) colonising abandoned wet grasslands can be done by cutting them from August to March. However, a common problem of this kind of action is their quick regeneration and re-growth, in particular *Salix* species, which show vigorous re-sprouting abilities. Regular mechanical cutting and/or use of herbicides on stumps are possible methods to limit the re-growth for maintaining open grasslands (Klimkowska *et al.* 2010b), but these methods are quite expensive for the former or questionable for the second as the most employed of these chemicals has now proved to have lethal impacts also on other organisms like amphibians (Relyea 2005). An efficient alternative on alluvial soils is to pull the stumps with a cable and a winch and, when the ground is sufficiently firm, to remove them with a grapple skidder, which can grab and tear them out (see Photos 2 to 5). Indeed, this allows to remove the root system from the ground and to prevent their re-growth. Moreover, this generates holes which can create small ponds for the aquatic fauna and flora. When *P. bistorta* is present in the vicinity, it can invade free spaces opened by the intervention and located above the water level, although this can be quite slow due to *P. bistorta*'s weak seed dispersal or strong concurrence by *Juncus*



Photo 1: Restoration mowing in July, with low bearing equipment, to reduce dominance by *Filipendula ulmaria*. Photo: A. Rauw

effusus (P. Lighezzolo, pers. obs.). Accordingly, seed sowing or rhizomes burying provides an efficient way to favour the food plant's recovery (see next section).

Restoration measures, like mowing, can have a potential important impact on populations of *L. belle* or other patrimonial species (see 'Habitat management' section, below). Consequently, there is a need to know which species are still represented on degraded sites to modulate restoration actions and mitigate their impact, notably by limiting measures to part of sites and spreading restoration over several years (rotation principle, see below). Therefore, preliminary inventories are essential on such sites before deciding an action plan.

HABITAT RE-CREATION

Under this expression, we consider the reconstitution of habitats on intensive grasslands or, more often, plots which have been planted since a long time (> 30 years) with exotic trees, like Spruce (*Picea abies*) or Hybrid Poplar (*Populus x canadensis*). Reconstitution includes two steps in reference to the Bakker and Londo (1998) classification: *environmental restoration* (of abiotic conditions) and *restoration management* (of biotic communities). This type of action is really needed in order to strengthen the habitat network and reconnect populations in many regions (linkage strategy, see above), and it constitutes



Photos 2 - 5: Violet Copper habitat restoration by removing willow scrubs (carrs) and tree groves, in four steps: willow or tree pulling with a cable and a winch (2), stumps removal with a grapple skidder (3), cleaning with a chainsaw (4), piling of branches that can be valued as a bio-fuel for electricity generation with biomass (5). Photos: P. Lighezzolo.

the key stone of any long-term conservation project, especially in the context of Western European fragmented landscapes and the changing climate (Lovejoy and Hannah 2005).

Alluvial soils planted with exotic tree plantations can quite easily be converted to humid grasslands by cutting the trees, windrowing branches and stumps, and then grinding them (see Photos 6 to 11). Rotovation of the upper soil layer with roots and stumps, followed by harrowing, can also be done to get a flat ground. It is crucial, however, as already stressed, not to compact soils too much to prevent encroachment by soft rushes (*Juncus effusus*). For this purpose, adapted machinery with low bearing or, better, horses are to be used to haul logs from the area.

Raising the water table may be desirable in some areas, which have been drained by ditches dug before plantation, at least when drainage is still effective and if the water quality is preserved (Klimkowska *et al.* 2010d). Indeed, rewetting with ground water may promote the development of target vegetation (Oomes *et al.* 1996). This can be done by plugging or filling up ditches.

Restoring flower-rich meadows from intensive mown grasslands and pastures requires to lower the nutrient level, especially phosphorus, present in the upper layer (Marrs 2002). The removal of topsoil is a radical but very effective way to achieve this goal on mineral soils (Tallowin and Smith 2001) as well as on peaty soils (Klimkowska *et al.* 2007). The



Photos 6 - 11: Humid grassland re-creation by cutting spruce plantations and cleaning the soil. (6) hauling branches with a grapple skidder, (7) windrowing branches and stumps with a bulldozer, (8) grinding stumps and woody debris with a forestry mulcher, (9) wood windrow which can then be milled to reduce its volume, (10) smashing the ground, (11) smoothing out the soil with a harrow before seed sowing or hay transfer, to allow subsequent mowing. Photos: P. Lighezzolo.

nutrient-rich soils removed can be piled in elongated mounds (at a maximum height of 1.5 metre) along the periphery of the site and planted with shrubs or trees. If the nutrient content of the upper layer is not too high, the area can be temporarily abandoned (one to ten years) or the ground can be milled and harrowed before introducing target vegetation seeds (see below) and light management, either rotational grazing or mowing.

Re-colonisation of restored areas by *P. bistorta* is of course essential to provide new habitats for the Violet Copper. This plant sometimes can re-colonise cleared soils very quickly, if still present in the herbaceous layer of the woods, but this seems a scarce event

in the Ardenne's massif. In many occasions, the reverse was observed, due to an absence, e.g. disappearance of the plant (rhizomes) or its propagules (seeds), in the restored plots, even when bistort is still present in adjacent areas. This comes probably from the plant traits of seed persistence in the soil and seed dispersal. Seed persistence in the soil is considered "transient", i.e. seed viability is < one year (Klimkowska *et al.* 2010a) (Longevity Index = 0.04)². Seed dispersal by wind has, to our knowledge, not been measured on bistort, but is likely to be weak according to releasing height (0.3 to 1.0 m)³, seed morphology and weight (Soons *et al.* 2004; Soons 2006). On the other hand, bistort seeds have rather good buoyancy (50% seeds floating at least 25 days in moving water) and are probably dispersed more efficiently by running waters during large alluvial flooding, although these events remain rare in wet grasslands (van den Broek *et al.* 2005). So, chances of spontaneous colonisation by bistort after soil disturbance (tilling or topsoil removal) appears to be very low due to seed limitation. Two methods can then be used to promote its return: rhizome pieces burying and / or seed sowing. The first method gave fruitful results on several sites in Ardenne (Parkinson D., pers. comm.) and in Lotharingy (Cavelier E., pers. obs.). This method is quite easy to implement if applied in early spring at a time when soils are waterlogged, although collection and planting can be time-consuming (E. Cavelier and P. Lighezzolo, pers. obs.). However, plants appeared only the second year after planting. The second method has only recently been tested by hand on different plots in Lotharingy. Seed collection and sowing is easier and faster but, at this stage, the results seem less convincing, regarding the low rate of germination obtained (Cavelier E., pers. obs.).

Transfer of green (undried) hay is another promising way to reconstitute *P. bistorta* meadows, but this method, now regularly used in ecological restoration projects (Donath *et al.* 2007; Hedberg and Kotowski 2010; Kiehl *et al.* 2010; Klimkowska *et al.* 2010c), has not been tempted in Ardenne and Lotharingy wet grasslands, at our knowledge. It will be initiated during the Belgian Life+ butterfly project on several plots. Time of cutting will be evidently crucial to get the maximal bistort seed set, most likely around the second half of June. The appropriate amount of applied hay has still to be determined, but an area ratio of 1:1 to 3:1 between donor and receptor sites seems a good a priori starting point, according to the productivity of *P. bistorta* wet grasslands (Kiehl *et al.* 2010).

To restore suitable new habitats for the Violet Copper, bistort wet grasslands need also to be surrounded or dotted with thickets, hedges, tree groves or wooded stands. If no ligneous vegetation adjacent to the new restored plots exist, plantations of native trees and shrubs can be carried out, especially along the western and northern margins, to create sheltered sunny edges. A practical, efficient and inexpensive method is to plant willow (preferably *Salix aurita* in Ardenne) and aspen stem cuttings, of about 30–60 cm length, deeply into moist soils in late winter, spring or fall (Gage and Cooper 2004; Pezeshki *et al.* 2007; Radtke *et al.* 2011). Plantation of other complementary species has also to be promoted, particularly trees with abundant flowering, used as nectar sources by adults

² LEDA Traitbase (<http://www.leda-traitbase.org>) (Knevel *et al.* 2003)

³ LEDA Traitbase (<http://www.leda-traitbase.org>) (Knevel *et al.* 2003)

of *L. belle*, such as Mountain Ash (*Sorbus aucuparia*), White Beam (*Sorbus aria*), Crab Apple (*Malus sylvestris*), Hawthorn (*Crataegus monogyna*), Blackthorn (*Prunus spinosa*) or Alder Buckthorn (*Frangula alnus*) (Goffart and Waeyenbergh 1994; Goffart *et al.* 2001).

HABITAT MANAGEMENT

The transitional character of *L. belle* habitats requires a management to prevent spontaneous complete reforestation and to preserve biotopes in an adequate state for the species on the long term. Three main methods have been (or can be) proposed: mowing, grazing or burning. However, these practices may have deleterious impacts on butterfly populations and they must be appreciated beforehand to ensure their wise use.

It has been noticed that *L. belle* rarely occurs on annually mown meadows and prefers abandoned grasslands or wetlands, which are irregularly mown (Fischer *et al.* 1999; Nunner 2006; Steiner *et al.* 2006; Bauerfeind *et al.* 2009). For a better understanding, the potential effects of mowing on *L. belle* populations were studied in two distinct experiments conducted in an abandoned wet grassland of the Ardenne's massif (Goffart *et al.* 2001, 2010): one to estimate the impact of mowing on larval stages, by comparing numbers of emerging adults in mown plots (in summer – from the end of June to the beginning of August – or in autumn – October) with those in not mown control plots (by means of emergence traps); the second to measure the corresponding attractiveness of mown plots and control plots on adults during the next season.

The impact of mowing on larval stages was different according to the timing of mowing with a significant difference between summer and autumn intervention: summer mowing seemed harmful while autumn mowing showed no influence on the probability of emergence of adult butterflies. This difference can be explained by the pre-imaginal life-cycle: caterpillars are indeed present on bistort leaves until the beginning of August, being vulnerable to hay export, followed by pupae in the upper soil layer afterward, where they could escape to haying.

In the other experiment, *L. belle* adults were significantly more attracted towards mown plots. This was clearly linked to the higher density of some nectar flowers like *Cardamine pratensis* (Goffart *et al.* 2001), as the majority of the adults butterflies were observed feeding on them. Nevertheless, this attraction did not result in higher numbers of eggs laid in the same plots, but rather a (non-significant, but over the years consistent) trend for a reduced number in mown plots compared with abandoned plots (Goffart *et al.* 2001). This last observation is rather fortunate as it tempers the eventuality of an “ecological trapping effect” (*sensu* Shlaepfer *et al.* 2002; Battin 2004) of mown vegetation, which would attract egg-laying in plots where survival of eggs or caterpillars would be strongly reduced afterwards.

Studies in Germany and Switzerland suggested that extensive grazing could be an alternative method of management as *L. belle* populations were found on several grazed sites (Fischer *et al.* 1999; Steiner *et al.* 2006; Bauerfeind *et al.* 2009). This insight has been evaluated more precisely in the Ardenne's massif through field experiments seeking to

measure the impact of light grazing regimes on several nature reserves managed with hardy breeds cows (Highland Cattle and Galloways) or ponies (Goffart *et al.* 2010). Transects were made on grazed plots and abandoned (control) plots to evaluate butterfly abundance. Three distinct forms of grazing were tested: (1) continuous grazing, livestock being present all the year round; (2) seasonal grazing, livestock being present only during late summer and autumn; and (3) alternate grazing, livestock being present every other year (for 3 to 9 months). After at least five years of management application, *L. helle* has been found on every grazed plots but abundance was sometimes significantly reduced to compare with control plots depending on grazing modality. The loss proved to be higher (-50%) in the case of seasonal grazing. When looking at both *L. helle* and *B. eunomia* populations together, the best regime appeared to be alternate grazing.

Burning is also a potential method to manage open communities (Swengel 1996; Vogel *et al.* 2007), including bistort humid grasslands. However, at our knowledge, the impact of fire has not been studied on *L. helle* populations and habitats. Early spring (quick) burning is actually used in Belgian Ardenne to manage one big military camp of about 2500 ha (Elsenborn) where the Violet Copper is present. It is carried out in a mosaic basis, with about 100-200 ha of moors being burned every year (Pirard 2010, 2011). Bistort humid grasslands are also rejuvenated in the process. However, this management maintains a virtually treeless large open landscape, inauspicious for *L. helle*. Indeed, places where the butterfly thrives in this area are restricted to partially wooded (unburned) dales near the margins of the military camp and permanent non-fire refuges seem essential for the survival of the species on this site (Swengel and Swengel 2007). Regardless to its potential impact on Violet Copper populations (especially pupae in early spring), patchy and quick burning appears therefore rather inappropriate to manage habitats of this butterfly because this method is difficult to use and control in confined clearings and is likely to destroy the willow thickets and birch groves.

To conclude, the intrinsic difficulty with habitat management for the Violet Copper comes from the binary and transitional character of its habitats (openings and forest) and from the sensitivity of the species to management methods. Therefore, a compromise between management and abandonment has to be found. Recommendations following these observations were already outlined and sometimes discussed at greater length elsewhere (Fischer *et al.* 1999; Goffart *et al.* 2001, 2010; Bauerfeind *et al.* 2009; Van Swaay *et al.* 2012). We can summarize them as follows⁴:

- two options exists basically to maintain a low-intensity management, rotational mowing or grazing, with a pluriannual regime (2 – 6 years) that leaves unmanaged areas every year;

⁴ note that these recommendations take also into consideration the requirements of many other patrimonial butterfly species thriving in the same biotopes (Dumont *et al.* 1997, Goffart *et al.* 2001, 2010); they are also appropriate for many other organisms, whether plants, insects, reptiles or birds... (Delescaille 2000, Humbert *et al.* 2012)

- mowing should best be done in autumn after pupation; as it does not match local farmers economic requirements, this method is in practice restricted to small plots (< 1 ha) in nature reserves;
- grazing should be applied with very low stocking rates (<0.2 Livestock Units ha⁻¹), avoiding preferably sensitive parts of the butterfly life cycle (April to July); this method is best suited for large areas (> 10 ha) as the risk of overgrazing with detrimental impacts on butterfly populations is higher on small areas.

An alternative to regular management would be a temporary abandonment of habitats, followed by rejuvenation through selective tree cutting, shrub out-rooting and meadow restoration (see 'habitat restoration' section above), with long cycles (10-50 years), but costs and benefits of this strategy should be evaluated and compared to that of regular management.

FIRST RESULTS AND FUTURE PROSPECTS

At this stage of the project (after 4 years of 6), the results are promising as more than 190 hectares in all have already been restored and/or managed for *L. belle* in the three concerned areas, and about 110 additional hectares are furthermore programmed so that the initial objective of 250 hectares will be largely met and exceeded (> 20%) at the end of the project (December 2014). However, we have to underline that, after interventions (the costs of which are given in the appendix 1, for information of managers), the full development of suitable habitats will take time, probably from five to 20 years, depending on the initial situations of the particular plots, and the same is likely afterward for the colonization by our target butterfly. So, it will not be possible to evaluate the full impact/success of this project on *L. belle* populations before ten to 20 years (Woodcock *et al.* 2012).

We tried to base as much as possible restoration actions on scientific evidence to obtain the best possible results, but also identified some gaps in the knowledge, which should be filled in the future. We believe that the three most critical issues for further work are:

- to estimate the maximal distance between habitat patches to allow effective dispersal and sufficient gene flow and landscape features impacting gene flow; this requires genetic studies at the landscape-scale to assess gene flow intensity and direction among populations (Baguette *et al.* 2013).
- to assess the minimal size and structure of a habitat network to support a long-term viable *L. belle* population; this can be addressed by PVA or MVM simulations (Schtickzelle and Baguette 2009);
- to evaluate the best methods and modalities, in terms of efficiency and convenience, to reintroduce *L. belle* in newly restored plots, whether by rhizome pieces burying or seed sowing and at which density.

Another interesting topic would be to assess the impact of tree-cutting on *L. belle* populations. Since trees are important for different activities of adults like nectaring, mate-searching (territorial defense), sun-basking and roosting behaviour, the suppression of trees could have an impact on the distribution of adults on sites at the least and probably also on butterfly total numbers if lengths of arboreal edges fall under a certain level. This could be determined through full-scale field experiments, by comparing adult and egg/larvae distributions before and after wood cutting in or along *L. belle* habitats.

While some gaps still exist in our knowledge, scientific evidence seemed satisfactory in many respects to launch a restoration project in favour of *L. belle* populations. We are convinced that, in many cases of conservation concern, we know enough to take action and that action and science can be conducted in parallel so that, in this way, they could feed each other (Arlettaz *et al.* 2010).

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Appendix 1: Costs ranges for land purchasing, habitat management, restoration or re-creation in Belgian Ardenne (data: Butterfly Life Project).

Objective	Action	Minimal cost (in Euros)*	Maximal cost (in Euros)*	Remarks
Land purchasing	Land purchasing	1 970 €	7 500 €	Mean = 5 300 € (land price, excluding wood)
Habitat management	Rotational mowing	0	300 €	Costs can be reduced when the job is done by contracted farmers (under agri-environmental schemes).
	Light grazing			
	Fencing	4 € per meter	8.2 € per meter	With 4 barbed wire
	Livestock	250 €	375 €	Considering a stocking rate of 0.2 UGB/ha and that one Galloway cow ~ 0.8 UGB
	Complementary feeding	75 €	100 €	During winter time (on «parking» meadows)
	Veterinary costs	?	?	Low cost due to livestock hardiness and low stocking rates!
	Episodic clearing / grinding	800 €	3 700 €	
Habitat restoration	Tree cutting	1 000 €	1 800 €	Shearing and windrowing
	Windrowing	600 €	2 200 €	Depending on the presence of windfall
	Willow (tree) pulling	4 300 €	7 500 €	80 - 100 EUR per hour
	Rank vegetation mulching or milling	400 €	1 950 €	About 175 EUR per hour
Habitat re-creation	Tree cutting - scrub removal	0	5 200 €	Timber prices can offset operating costs, except with scrub vegetation
	Grinding of woody debris	800 €	3 700 €	To produce woodchips
	Stump grinding	2 850 €		About 5 € by stump
	Topsoil removal	2 500 €	4 000 €	
	Seed sowing	1 000 €	2 000 €	Including seed harvest + sorting
	Hay harvesting, transfer and spreading	2 000 €	3 000 €	

*All costs are given per hectare, except where indicated

Caught in a trap: How to preserve a post-glacial relict species in secondary habitats?

Klaus Fischer*, Eva Schubert & Johannes Limberg

University of Greifswald, Zoological Institute and Museum, D-17489 Greifswald, Germany

*Corresponding author: Klaus Fischer, Department of Animal Ecology, Zoological Institute and Museum, University of Greifswald, Johann-Sebastian-Bach-Str. 11/12, D-17489 Greifswald, Germany, E-Mail: klaus.fischer@uni-greifswald.de

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Abstract

The Violet Copper *Lycaena helle* is one of the rarest butterflies of Europe, where it is largely considered a post-glacial relict species. Owing to strong population declines throughout its European range, targeted conservation measures are needed to avoid further local and regional extinctions. This is even more important since anthropogenic climate change will most likely pose additional challenges for the survival of *L. helle*. Against this background, we here present data on habitat requirements, population structure and endangerment of *L. helle*, based on univoltine mid-altitude populations (Westerwald mountain range, Germany) and a bivoltine low-altitude population (north-east Germany). *L. helle* can be characterized as a hygrophilous habitat specialist, which in mountain ranges primarily inhabits secondary habitats, namely abandoned moist meadows (> 85% of all populations in the Westerwald mountain range), while hay meadows are used in north-eastern Germany. In the Westerwald mountain range, occupied patches are characterized by a relatively high altitude above sea level, a large patch size, a high connectivity, a high ground coverage of its larval host plant and of shrubs, but a relatively low ground coverage of nitrophilic plants. Regarding the vegetation structure, similar results were obtained from the low-altitude population, preferring stands with a low vegetation density and height, but with a high abundance of the larval host-plant and of flowers for adult feeding. *L. helle* is particularly sensitive to environmental changes due to its (1) specialized habitat requirements, (2) low dispersal ability, and (3) its comparably low fecundity. In the Westerwald mountain range, reforestation of fallow wetland was the most important threat to *L. helle* populations in the early 1990ies. Currently, eutrophication and

overgrowth of larval habitats by tall herb and nitrophilic plant communities are the most severe threats. To halt these processes, maintaining / restoring a high water table and appropriate management actions are of crucial importance. Unfortunately, *L. helle* seems to be extremely sensitive to management. Against this background, we discuss different management options, highlighting the different needs of uni- versus bivoltine populations and of different habitat types.

LYCAENA HELLE: A POST-GLACIAL RELICT SPECIES

The Violet Copper *Lycaena helle* (Denis and Schiffermüller 1775) is a boreo-montane lycaenid butterfly with a vast distribution range, extending from the Picos de Europa in Spain through to the easternmost parts of Siberia and China (Tolman and Lewington 1997; Gorbunov and Kosterin 2003; Bozano 2004). In contrast to its rather continuous distribution in the taiga zone (Gorbunov and Kosterin 2003), populations are highly fragmented in western and central Europe, where *L. helle* is considered a post-glacial relict species (Meyer 1982a, 1982b; Buszko 1997; Eliasson et al. 2005; Habel et al. 2011). During the last glacial period, *L. helle* was probably widely distributed throughout Europe, but retreated northward and uphill (towards mountain areas), respectively, to escape the increasing temperatures during the postglacial period (Habel et al. 2010b, 2011).

Therefore, the species nowadays is restricted in western and central Europe to some watersheds of high (Alps, Pyrenees, Carpathians) as well as lower mountain ranges (e.g. Ardennes and Eifel, Westerwald), with only one known extant lowland population in north-eastern Germany (Meyer 1980, 1982a, 1982b; Ebert and Rennwald 1993; Hennicke 1996; Fischer et al. 1999; Habel et al. 2010a; Wachlin 2011). In central and western Europe, *L. helle* has likely been a local species restricted to relatively few localities for several thousand years already (Meyer 1982a, 1982b). Consequently, the remnant populations of different mountain ranges are genetically strongly differentiated, thus comprising evolutionary unique entities characterized by endemic private alleles (Habel et al. 2010b, 2011).

Due to its local occurrence combined with local and regional extinctions, *L. helle* is currently one of the rarest butterflies of Europe (Van Swaay and Warren 1999; Bauerfeind et al. 2009; Van Swaay et al. 2010). In the Westerwald mountain range, for instance, 50 out of 138 local populations went extinct from 1994 to 2004, while only 9 colonization events could be documented in the same time period (Bauerfeind et al. 2009). The species is considered endangered even at the global scale because of strong population declines throughout its European range, including Fennoscandia, over past decades (Van Swaay et al. 2010). In the recent past, habitat loss comprised the singly most important threat to *L. helle*, mainly due to the widespread deterioration of wetlands, e.g. through the intensification of agricultural land use, land drainage and reforestation (Meyer 1980; Fischer et al. 1999; Van Swaay and Warren 1999; Nunner 2006; Van Swaay et al. 2010). Anthropogenic climate change, including global warming in association with increasing frequencies of extreme weather events such as droughts and

heat waves, is likely to largely exaggerate detrimental effects on population persistence, posing a severe threat to the longer-term survival of this species at its western range margins (Habel et al. 2010b, 2011).

OUTLINE

Based on the threats detailed above, targeted conservation measures are evidently needed in order to avoid further local extinctions and eventually the complete loss of *L. belle* populations from its currently occupied habitats at lower altitudes. Therefore, we focus here on the habitat requirements, population structure and endangerment of *L. belle* in the Westerwald mountain range (western Germany), comprising a major stronghold of this species in central Europe (Fischer et al. 1999; Bauerfeind et al. 2009). We will compare the findings obtained from these univoltine mid-altitude populations (500–650 m a.s.l.) to the results on habitat use from the only German lowland population, in order to (1) draw more general conclusions regarding management options and (2) to highlight the different conservation needs warranted by the specific habitat used. The latter lowland population occurs in a floodplain near the Baltic Sea in northeast Germany, and has two generations per year as the ones in the southern Baltic and Siberia (Hennicke 1996; Wachlin 2011).

HABITAT REQUIREMENTS

The habitat needs of any species can be characterized as a specific set of complementary resources consisting of consumables (e.g. host-plants) and utilities (e.g. perch structures; Bauerfeind et al. 2009). Abundance and availability of these resources determine the suitability and quality of any given patch for the focal species, and is thus of crucial importance for population occurrence and persistence (Maes et al. 2006; Dennis and Hardy 2007). The most important resource for *L. belle* in central Europe is its only larval food plant, *Polygonum bistorta*, a high abundance of which being crucial (Fischer et al. 1999; Nunner 2006).

For central Europe, *L. belle* in general terms can be characterized as a hygrophilic habitat specialist restricted to humid and cool, relatively nutrient-poor ‘grassland’ habitats (Fischer et al. 1999; Finger et al. 2009). Note in this context that, while the mesoclimatic conditions are typically cool (see above), the butterflies fly mainly in warm and sheltered microclimates such as pockets at forest edges and sunny forest openings (Fischer et al. 1999; Nunner 2006; Steiner et al. 2006; Turlure et al. 2009, see Chapter IV). Shrubs or trees that are also used as perching and sleeping sites or other wind shelter (rocks, ground depressions etc.) are an essential habitat requisite (Fischer et al. 1999; Nunner 2006; Steiner et al. 2006; Turlure et al. 2009), such that *L. belle* can be considered a species of open forest ecotones.

Natural habitats include forest clearings, fens, transition mires, peat bogs, springs, marshes, water-fringe vegetation, alpine meadows and boggy woods (Fischer et al. 1999; Biewald and Nunner 2006; Nunner 2006; Steiner et al. 2006; Turlure et al. 2009). Such natural habitats have been widely destroyed throughout Europe (Fischer et al. 1999). Consequently, the species currently inhabits in the first place secondary, semi-natural habitats, mainly unimproved, abandoned moist meadows (Meineke 1982; Fischer et al. 1999; Biewald and Nunner 2006; Nunner 2006; Goffart et al. 2010; Van Swaay et al. 2010). In the Westerwald mountain range, > 85% of all populations occur in such secondary habitats (Fig. 1).

A comparison of occupied versus vacant patches in this area revealed that patches harbouring *L. helle* populations were characterized by (1) a higher altitude above sea level, (2) a larger patch size, (3) a higher connectivity, (4) a higher ground coverage of its larval host-plant *P. bistorta*, of *Deschampsia caespitosa* (a grass species being regularly associated with *P. bistorta*), and of shrubs within patches, but (5) a lower ground coverage of nitrophilic plants (Bauerfeind et al. 2009). No difference though was found for flower abundance, which is in line with the concurrent literature. Imagines accept a wide variety of nectar plants in a rather opportunistic manner: more than 40 different plant species have been reported for central Europe (Hasselbach 1985; Ebert and Rennwald 1993; Fischer et al. 1999; Biewald and Nunner 2006).

Results similar to above were obtained for the only lowland population in Germany (Table 1). Here butterflies were found in both generations to prefer stands with a lower vegetation density and height, but with a higher abundance of their larval host plant and of flowers for adult feeding (Fig. 2; Table 1a). Similarly, larvae (first generation) and eggs (second generation) were found at places with a lower vegetation density and height, but with a higher host plant and flower abundance, though differences between the locations of immature stages and random control sites were less pronounced compared to adults (Table 1b). Egg counts in this population revealed that, on average, 45 ± 6 ($n = 19$) *L. helle* eggs were laid per 50 *P. bistorta* leaves, with a pronounced spatial variation. Per leave with eggs, on average 2.7 ± 1.6 eggs were deposited with a maximum of 19 eggs per leave. These numbers are very high as compared to e.g. Steiner et al. (2006) (see also Chapter IV), which may either result from a particularly high population density in our

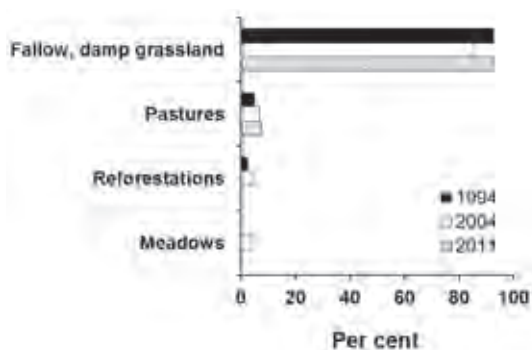


Figure 1: Land use in the habitats occupied by *Lycaena helle* in the Westerwald mountain range in the years 1994 ($n = 83$), 2004 ($n = 128$), and 2011 ($n = 41$). Data taken from Fischer et al. (1999), Bauerfeind et al. (2009), and Limberg et al. (2011).

study area, or, more likely, from a much lower abundance of the larval host plant. For instance, while the abundance of *P. bistorta* ranged between 12 and 26% ground coverage in north-eastern Germany (cf. Table 1), the average value for occupied habitats within the Westerwald mountain range was 36% (Bauerfeind et al. 2009).

Table 1: Habitat preferences of adults (a) and immature stages (b) in a lowland population of *Lycaena helle* in northeast Germany in 2007. For adults, 9 m² plots occupied by adults were compared to unoccupied plots of the same size (n = 25 each for the first and n = 20 each for the second generation). For larvae / eggs, 1 m² plots with a larva / egg were compared to random 1 m² plots containing a larval host plant, with control plots being located ca. 10 m to the north of the former plots (n = 30 each per generation). Vegetation density was classified in three categories (low, intermediate, high) for adults and was measured in % in horizontal projection for immature stages (the mean of three measurements is given here). Vegetation height is given as the mean of 16 individual measurements for adults and as the mean of nine measurements for immature stages. The abundance of the larval host-plant *Polygonum bistorta* was assessed as the percentage of ground covered by this plant. The number of flowers was counted per 9 m² and per 1 m² plot, respectively. Parameters were compared between occupied and control patches by Mann Whitney U-tests.

a)

Parameter	Occupied	Control	Z	p
First generation (adults)				
Vegetation density	1.8 ± 0.2	2.5 ± 0.2	2.74	0.006
Vegetation height [cm]	36 ± 1	49 ± 3	3.09	0.002
Abundance <i>P. bistorta</i> [%]	15 ± 2	4 ± 1	4.95	< 0.001
Flowers [n / 9 m ²]	50 ± 8	20 ± 5	3.44	0.001
Second generation (adults)				
Vegetation density	1.8 ± 0.1	2.7 ± 0.2	3.09	0.002
Vegetation height [cm]	45 ± 3	71 ± 6	3.58	< 0.001
Abundance <i>P. bistorta</i> [%]	12 ± 2	2 ± 1	5.13	< 0.001
Flowers [n / 9 m ²]	55 ± 13	35 ± 13	1.85	0.064

b)

Parameter	Occupied	Control	Z	P
First generation (larvae)				
Vegetation density [%]	32 ± 3	37 ± 4	1.27	0.204
Vegetation height [cm]	36 ± 1	45 ± 3	1.97	0.049
Abundance <i>P. bistorta</i> [%]	17 ± 1	11 ± 2	3.59	< 0.001
Flowers [n / 9 m ²]	23 ± 3	12 ± 2	2.89	0.004
Second generation (eggs)				
Vegetation density [%]	25 ± 3	50 ± 4	5.01	< 0.001
Vegetation height [cm]	34 ± 1	49 ± 2	5.20	< 0.001
Abundance <i>P. bistorta</i> [%]	26 ± 2	14 ± 1	3.64	< 0.001
Flowers [n / 9 m ²]	6 ± 1	8 ± 1	1.32	0.188

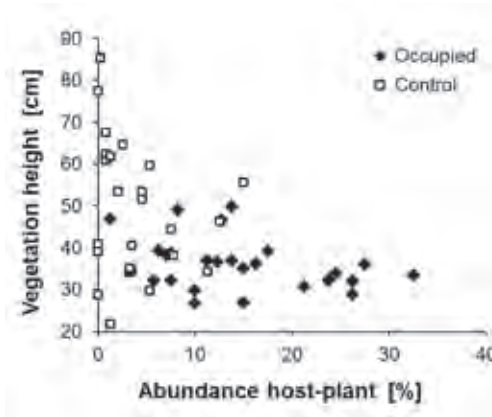


Figure 2: Scatter plot of vegetation height in relation to host plant abundance for 9 m² plots occupied by *Lycaena helle* compared to unoccupied control plots (n = 25 each). For details see Table 1.

DISPERSAL ABILITY: HOW TO REACH NEW HABITAT?

Although habitat quality is clearly of great importance for species occurrence and population survival (see above), it may not be sufficient to fully predict patch occupancy (e.g. Hanski 1994, 2001; Haynes et al. 2007; Binzenhöfer et al. 2008). Especially in modern fragmented landscapes, suitable habitats are often patchily distributed, such that a species' long-term persistence relies on habitat networks sufficiently close to allow for dispersal and thereby (re-)colonization (Dennis and Eales 1997; Hanski 1998, 2001; Haynes et al. 2007). In such networks, populations inhabiting small and/or isolated patches face an increased extinction risk as compared to large and/or less isolated patches (Hanski 1998). *L. helle* though is considered a sedentary species with an exceedingly low dispersal ability, severely hampering its capability to colonize habitat patches and therefore posing a challenge to its survival in fragmented European landscapes (Baguette and Nève 1994; Fischer et al. 1999; Fischer and Fiedler 2000; but Bachelard and Descimon 1999). This notion is supported by several lines of evidence as outlined below.

First, mark-recapture studies showed that average movement distances are very low (males: ca. 40 m; females ca. 60 m) and that the vast majority of movements is restricted to specific parts of the habitat, while exchange even between neighboring and well connected patches is very rare (Fischer et al. 1999; Fischer and Fiedler 2000). Movements between a capture and the consecutive recapture event covered in 89% of all cases less than 100 m (Fischer et al. 1999). Thus, dense forests as well as open meadows may serve as barriers to dispersal in this species (Fischer et al. 1999; Chuluunbaatar et al. 2009).

Second, the species occurs only in a fraction of patches and landscapes that seem suitable (Fischer et al. 1999; Biewald and Nunner 2006; Nunner 2006). Its occurrence is probably confined to areas with a long-standing habitat continuity, in which *L. helle* could successfully invade secondary habitat in close vicinity to (former) primary habitat (Nunner 2006). Concomitantly, studies in the Westerwald mountain range showed that patch connectivity is the strongest predictor for patch occupancy, followed by the abundance of the larval host plant and patch size (Bauerfeind et al. 2009).

Third, the species shows a remarkable morphological diversity throughout its European range, having resulted in the description of nine subspecies (Meyer 1982). Such differences are indicative of a lack of long-distance dispersal, which could be fully backed up by recent population genetic analyses. While genetic diversity was typically high within *L. belle* populations, populations of different mountain ranges are clearly genetically differentiated with private endemic alleles, confirming a strongly interrupted gene flow (Finger et al. 2009; Habel et al. 2010a, 2010b, 2010c, 2011). Comparably high genetic differentiation even within mountain ranges (e.g. Ardennes, Westerwald) further indicates a disruption of population connectivity and thus additionally stresses the species' low dispersal ability (Finger et al. 2009; Habel et al. 2011).

Taken together, these findings suggest that *L. belle* faces a comparably high extinction risk based on demographic and genetic stochasticity (Frankham 2005; Finger et al. 2009; Habel and Assmann 2010). The low dispersal ability of *L. belle* is of particular concern since niche modeling revealed that anthropogenic climate warming will cause a loss of the majority of currently occupied sites within central and western Europe, except for a few exclaves of potentially still suitable areas in the high altitudes of the Pyrenees, the Massif Central and the Alps (Habel and Assmann 2010; Habel et al. 2011).

THREATS TO *L. HELLE* SURVIVAL

Any given species' endangerment follows from a specific combination of inherent ecological attributes reflecting its vulnerability and (anthropogenic) driving forces posing concrete threats to its survival. *L. belle* has evidently a high inherent disposition for being endangered because of its (1) specialized habitat requirements, (2) low dispersal ability (see above) and, additionally, (3) its low fecundity resulting in a low potential to respond to environmental perturbations (Fischer 1998). The principal driving forces threatening population survival in *L. belle* are habitat loss and deterioration and in upcoming decades probably additionally rapid human-induced climate change.

An inventory in the Westerwald mountain range in 1994 revealed that the reforestation of habitats (fallow grassland) was the most frequent threat to *L. belle* populations, followed by secondary succession resulting in the replacement of larval habitats by tall herb communities, land drainage, grazing and finally mowing (Fig. 3a). For 21 (36 %) out of 58 habitats, no major threatening factors were found in 1994. Ten years later, reforestation did not play a major role anymore (Fig. 3b). This is caused by the fact that the reforested habitats still occupied in 1994 were completely lost by 2004, and that further reforestations of habitats did not occur at a larger scale anymore. Instead, eutrophication (caused by land drainage, peat mineralization and nitrogen depositions) and concomitant overgrowth of larval habitats by tall herb and nitrophilic plant communities turned out to be the most important threat (Fig. 3b). This is, at least partly, caused by the fact that the inhabited fallows are getting too old, most of which were set aside in the 1960ies already (Fischer et al. 1999). Further factors causing a deterioration of habitats were grazing, reforesta-

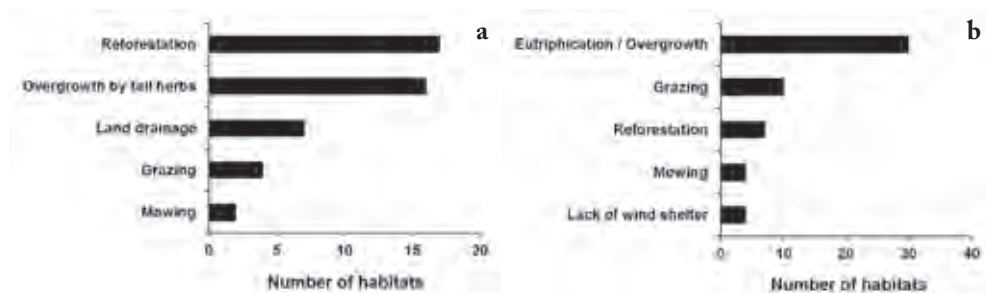


Figure 3: Causes for the deterioration of *Lycaena belle* habitats in the Westerwald mountain range in 1994 (a; $n = 37$) and in 2004 (b; $n = 55$). For a) more than one nomination per habitat is possible, while for b) only the most important threat is given. Data taken from Fischer et al. (1999) and Bauerfeind et al. (2009), respectively.

tion, mowing and insufficient wind shelter. In the 2004 inventory, no major reasons for concern were found for 73 (57 %) out of 128 habitats investigated (Bauerfeind et al. 2009).

The above trends were further corroborated in a recent inventory in 2011, which classified the habitat status for only 6 out of 41 *L. belle* (meta-)populations within the Westerwald mountain range as being ‘favorable’ for 21 as ‘fair’, and for 16 as being ‘poor’ (Table 2). The most important threat was the occurrence of disturbance indicators (i.e. nitrophilic tall herbs overgrowing larval habitat), followed by a lack of appropriate habitat management (i.e. typically a lack of any management) and land drainage. Insufficient wind shelter and shrub encroachment, in contrast, are of subordinate importance only. Currently, most habitats are very small accompanied by low numbers of individuals (Table 2).

The above data, covering a time span of 17 years in total, clearly indicate that currently the principal threat to *L. belle* habitats in the Westerwald area is secondary succession. This process is becoming ever more important since (1) the fallows are getting too old (see above) and (2) because the process is accelerating due to peat mineralization caused by (old) drainages and nitrogen depositions from other sources. The concomitant eutrophication results in a fairly quick replacement of *P. bistorta* communities by tall herbs without management actions.

The above findings are in broad agreement with others from central Europe, mentioning the following threats (e.g. Meyer 1980; Van Swaay and Warren 1999; Biewald and Nunner 2006; Nunner 2006; Van Swaay et al. 2010): Habitat loss, deterioration and fragmentation through agricultural intensification and changes in land use, reclaim of fallow land, land drainage, reforestation of fallow land, encroachment and overgrowth of larval habitats by tall herbs, shrubs and finally trees in secondary habitat due to succession, nutrient accumulation and eutrophication caused by mineralization of peat and atmospheric depositions. Further, current climate change poses a severe threat to *L. belle* survival in central Europe as highlighted above (Habel and Assmann 2010; Habel et al. 2011). Note here that range shifts to higher latitudes or altitudes are largely restricted to alpine populations, while populations from low mountain ranges will hardly be able to reach (future) suitable habitat (Parmesan and Yohe 2003; Finger et al. 2009).

Table 2: Habitat and population status as well as threatening factors for 41 *Lycaena belle* (meta-) populations in the Westerwald mountain range in 2011. Data taken from Limberg et al. (2011). Qualifiers are used for each parameter to indicate a 'favourable', 'fair' or 'poor' status.

Parameter	Qualifiers	Favorable	Fair	Poor
Patch size [ha]	> 5 ha; 1-5 ha; < 1 ha	2	19	20
Number of individuals per day	> 20; 10-20; < 10	26	12	3
Larval host plant [%]	> 60%; 10-60%; < 10%	1	38	2
Wind shelter	optimal; sufficient; sparse	14	26	1
Disturbance indicators [%]	< 5%; 5-25%; > 25%	7	11	23
Impact of drainage	minor/no; medium; strong	7	30	4
Shrub encroachment [%]	< 20%; 20-30%; > 30%	35	5	1
Habitat management	optimal; fair; insufficient	3	23	15
Overall evaluation		6	19	16

MANAGEMENT OPTIONS: DIFFERENT HABITATS WITH DIFFERENT NEEDS

According to the principle drivers of current and future population declines, conservation management should aim at (1) restoring and maintaining habitat quality and connectivity and at (2) buffering detrimental effects of climate change. Regarding the former, the principal challenge is that *L. belle* is currently largely dependent on instable, semi-natural habitat, namely fallow damp grassland surrounded by trees and shrubs (see above; cf. Fig. 1). Evidently, maintaining such habitat requires some sort of management to halt secondary succession, which, however, is barely tolerated by *L. belle*, for largely unknown reasons being extremely sensitive to management. For instance, regularly mown hay meadows do not comprise a suitable habitat for mountain populations of *L. belle* (Fischer et al. 1999; Steiner et al. 2006; Bauerfeind et al. 2009; Goffart et al. 2010; but see below). Perhaps, this sensitivity is caused by the need for a well-structured litter layer for overwintering pupae, which already pupate in summer (Steiner et al. 2006). Consequently, *L. belle* seems to be caught in a trap: this sedentary specialist species with low dispersal ability and fecundity is adapted to rather stable and continuous habitats (bogs within the taiga zone), but now mainly occurs in anthropogenic secondary habitat of relatively high dynamics. Unfortunately, large-scale restoration of primary habitat seems unrealistic at least for large parts of its current European range.

Against this background, which management options do we have? There seems to be general agreement that a mosaic-like, rotational mowing system with mowing intervals of typically several years (e.g. 3-5) and while sparing structures providing shelter (e.g. bushes) seems most appropriate (Fischer et al. 1999; Biewald and Nunner 2006; Nunner 2006; Goffart et al. 2010). Sparing the latter structures though does not exclude a partial removal of encroaching shrubs if necessary. Mowing should preferably take place in autumn (Goffart et al. 2010). Such a mowing system would maintain open fallow stages of moist grassland, while allowing for the colonization of recently mown areas from adjacent stands.

These regimes, however, need to consider the productivity of the habitat in question. While for nutrient-poor, stable fallows no or only sporadic management is sufficient, nutrient-rich stands may need to be mown 1–2 times per year. While the latter will likely eradicate univoltine mountain populations, thus exclusively comprising a means to develop new larval habitat on degraded stands, the bivoltine north-eastern population seems to be much more tolerant regarding management activities. The habitats occupied here are actually hay meadows, with their persistence relying on regular management due to a very high productivity. Without management, *L. helle* habitat here would be quickly replaced by tall herb, sedge and rush communities. This stresses the importance of considering the specific situation for management decisions.

We believe that the obvious difference regarding management tolerance between mono- and bivoltine populations has a biological explanation. We assume that, given the species' low fecundity, bivoltine populations are able to compensate losses through management (at least to some extent), while monovoltine ones are not. Note that the population size in north-eastern Germany largely depends on appropriate management actions (Wachlin 2011). Here, the second generation butterflies are able to recolonize stands that have been mown earlier the same year, thus allowing for a much greater flexibility compared to monovoltine populations.

On principle, grazing may comprise a cost-effective alternative to mowing, especially since the above mowing systems do not produce agricultural added value (Goffart et al. 2010). However, caution is necessary for several reasons. First, many habitats occupied by *L. helle* are unsuitable for grazing due to deep, boggy soils. Second and more importantly, grazing may have strong detrimental effects on population survival (Wipking et al. 2007; Falkenhahn 2008), although some populations seem to occur on pastures (Fischer et al. 1999; Steiner et al. 2006). The latter cases involve large-scale grazing systems with low grazing intensity, in which butterflies seem to rely on fallow-like, under-grazed structures such as moist depressions, springs or water-fringe vegetation (Fischer et al. 1999; Steiner et al. 2006). Even though eggs were occasionally deposited also within grazed areas in the Swiss Alps (Steiner et al. 2006), successful development at such places has not been documented and seems questionable. Note that *L. helle* females follow a risk-spreading strategy during oviposition, such that eggs can be regularly found at atypical places (Meyer and Helming 1994; Nunner 2006).

As the pace of habitat deterioration depends on nutrient availability (with high levels accelerating succession rates), nitrogen input into habitats should be minimized. An important issue here is the maintenance or restoration of a high water table, in order to avoid the mineralization of peat. High water tables may further, at least to some extent, buffer detrimental effects of global warming, as high soil humidity reduces thermal maxima and air humidity minima. The capacity of such measures to halt habitat deterioration and to buffer effects of extreme weather events have to be addressed in future research.

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Why protecting relict populations?

Jan Christian Habel^{1*} & Thomas Schmitt^{2,3}

¹Terrestrial Ecology Research Group, Department of Ecology and Ecosystem Management, Technische Universität München, D-85350 Freising-Weihenstephan, Germany

²Department of Biogeography, Trier University, D-54296 Trier, Germany

³Senckenberg German Entomological Institute, D-15374 Müncheberg, Germany

*Corresponding author: Jan Christian Habel, Terrestrial Ecology Research Group, Department of Ecology and Ecosystem Management, Technische Universität München, Hans-Carl-von-Carlowitz-Platz 2, D-85350 Freising-Weihenstephan, Email: janchristianhabel@gmx.de

ABUNDANCE DISPARITIES ACROSS DISTRIBUTION RANGES

In almost all cases, the distribution of a species consists of a core area and the distribution margin (with the sole exception of globally distributed taxa). Both parts of a species' distribution have typical characteristics: the population structure in the core area is mostly homogeneous and consists of large and often interconnected populations. On the contrary, the population structure at the distribution edge is often characterised by a reduction in the species' abundance and consists of small populations, which often occur in geographic isolation (Hanski 1999; Hampe and Petit 2005). These two opposite settings have led to diverging evolutionary processes within a species' distribution range (Melbourne and Hastings 2008).

Populations in the core of the distribution range in many cases show higher genetic variability (many alleles, high levels of heterozygosity) than at the margin. This high level of genetic diversity is maintained by large and interconnected populations, a situation which leads to an equilibration at this high level of variability accompanied by a low degree of genetic differentiation among populations (i.e. low genetic distances, F_{ST} etc.). This genetic pattern is strongly influenced by the low impact of population fluctuations and the hereon resulting bottlenecks, which, if happening at all, easily can be equilibrated from other nearby populations (Melbourne and Hastings 2008).

Populations from the distribution edges, in contrast, are mostly characterised by lower levels of variability among individuals within populations and a comparatively high level of differentiation among populations. This pattern is the consequence of small population sizes and low interconnectivity. This reinforces population fluctuations with their subsequent drift effects resulting in losses of intraspecific variability and increases in the degree of differentiation among local populations (Hampe and Petit 2005). Fur-

thermore, low interconnectivity reduces or even impedes individual exchanges among populations, hereby precluding a homogenisation of populations at the local and regional scale after stochastic events.

These biogeographic facts raise important questions for conservation concepts. One of the key-questions might be which of these two areas should be of prime interest in conservation management. Protecting the populations from the core of the distribution, we retain the main proportion of the overall variability as well as intact, viable population networks. In contrast, populations from the margin are often characterised by reduced within-taxon diversity (morphological features, alleles numbers or degree of heterozygosity), but strong differentiation among local occurrences (Hampe and Petit 2005), in some of the cases even representing endemic characteristics (e.g. alleles exclusively occurring in one local population, behavioural traits restricted to a limited geographic area). Therefore, we can conserve high diversity and viability in the core areas, and local uniqueness and specific adaptations in isolated remnant populations at the range margin.

CORE VERSUS MARGIN

As financial resources are limited for conservation issues (unfortunately considerably more limited than resources for saving banks or making war), we are forced to prioritize and to decide which populations, species, ecosystems and areas should be the main focus of conservation activities. Various conservation strategies were developed focusing on the protection of areas with extraordinarily high conservation value like centres of endemism (Myers et al. 2000), or highly endangered species (Rodrigues et al. 2006). In the majority of cases, the resulting debates on the prioritization are rather subjective and each scientist and conservationist might have an individually diverging priority list, depending e.g. being an entomologist or a mammalogist, or being interested in the tropics or the marine realm. In the case of the Violet Copper, we 'only' have to select between two regions of the distribution range:

- (i) In the core of a distribution, populations mostly show a comparatively high general variability. The conservation of this high variability might be of relevance if conservation management is intended to preserve the most viable individuals and populations, which are assumed to be most appropriate to successfully react on environmental changes, as climatic modifications, new habitat structures or novel interactions among taxa (Meffe and Groom 2006; Frankham et al. 2010). In the long run, large population networks and high species abundance are commonly assumed to guarantee a much higher stability than small and isolated populations do. Thus, the conservation of such core area occurrences might be more expedient than populations being on the brink of extinction at the range's margin. Several conservation strategies further underline an outstanding responsibility of countries for such species, which have their distribution cores located within a respective country (Meffe and Groom 2006). For *L. belle*, this concept would imply a clear

concentration of conservation activities on the central Palaearctic region (i.e. Siberia), the core of the butterfly's distribution.

- (ii) The western distribution margin of *L. belle* is located in the western Palaearctic, where almost all of the studies described in this book were performed; only the biogeographic chapter VI also includes specimens from Siberia. In contrast to the core area, the conservation of these small and isolated relict populations at the species' western distribution fringe are of high relevance when focusing on the preservation of local uniqueness (morphologic, behavioural, genetic), which evolved due to long-term isolation (here most probably during the late Pleistocene) as analysed and indicated for *L. belle* based on morphologic and genetic traits (Meyer 1981a, 1981b, 1982; Finger et al. 2009; Habel et al. 2010, 2011a, 2011b; see also chapters VI–VIII of this book). Giving consideration to the protection of local occurrences with specific unique characteristics was emphasized when Moritz et al. (1994) developed their concept of Evolutionary Significant Units (ESUs) covering the entire evolutionary legacy from the past, but also to guarantee for potential adaptations in the future. The conservation of such isolated occurrences may ensure the preservation of all local adaptations and thus of the entire variability. This concept would be of special importance if these populations are located at a putative leading edge of the distribution range from where future expansions might start. In the same time, such marginal populations and population groups are, of course, mostly isolated and small and, consequently, characterised by frequent and strong population fluctuations due to stochastic processes, the hereon resulting drift effects and subsequently limited variability. If changes in environmental conditions occur, the individuals from such impoverished populations are assumed to be less capable to successfully react on fast environmental changes (e.g. climate, land-use) and thus might be threatened by extinction (Frankham et al. 2010). Furthermore, populations at the distribution edge often suffer from delayed fallout effects (i.e. the species still exist, but the habitat conditions are no longer suitable for the respective taxon and thus local extinction can be predicted for the near future). Such populations being at the brink of extinction only kept alive due to (i) permanent immigration from neighbouring populations, (a phenomenon called rescue effect), or (ii) by rather artificial, often very cost-intensive conservation interventions (mostly rather non-fenced zoological or botanical gardens).

PRIORITIZING CONSERVATION EFFORTS

Against the view of many classical conservationists, conservation priority for a species as a whole should be given to populations, which will be able to persist in the long run, even independently from permanent conservation activities. This goal will be reached by establishing and protecting large population networks located in the core of a distribution range. On the contrary, isolated populations at the edge of the distribution might


need permanent support to persist over time. Individuals from the core might present a major part of the intraspecific variability. Thus, we can assume that, if environmental conditions are changing, we might have many individuals in viable core area populations with the pre-requisite to adapt rapidly on changing conditions, hereby being pivotal for the future survival of the species as an evolutionary unit.

This book on *L. helle* represents a compilation of contributions conducted by applying manifold methods. The isolated satellite populations on which the contributions of this book are focusing are especially of high value for understanding of evolution processes taking place in the wake of long-term isolation and limited population sizes, like environmental stress, local adaptations and genetic drift effects. The analysed topics are of particular interest in an evolutionary and ecological point of view. However, the western Palaearctic actually is the westernmost range margin of this butterfly, and, if the climate warming continues, these populations, or at least their large majority, might rapidly vanish and the species' distribution might inevitably contract north- and eastwards (Habel et al. 2011b). Despite the butterfly is listed as highly endangered in most of the European countries and is furthermore listed on the appendices of the Habitats Directive, exclusive species specific conservation measures for *L. helle* might be adjusted according to the global distribution situation and the biogeography of this species, by e.g. downgrading the IUCN categories accordingly (see Gärdenfors et al. 2001; Rodrigues et al. 2006). In our eyes, it might be a wise decision to canalize the limited financial resources for conservation issues maybe more to threatened species and species assemblages for which we have the highest responsibility by providing excellent habitat conditions for their core distribution populations. Let us enjoy on that "*Jewel in the mist*" as long as we still can find it somewhere in the marshy *Polygonum bistorta* meadows somewhere in higher elevations with moist and cool climatic conditions, before its distribution might finally retreat eastwards to northeastern Europe and the Russia bogs of the taiga belt by responding to the current climate warming.

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The background image is a photograph of a lush, green mountain landscape. In the foreground, there are dense, low-lying green plants with broad leaves. A small, clear stream flows through the middle ground. The background shows steep, forested slopes under a thick layer of mist or low clouds, creating a sense of depth and atmosphere.

This book is a compilation of 19 contributions about biogeography, ecology and conservation of the Violet Copper *Lycaena helle*. Although this volume focuses on a single butterfly species, this taxon stands as a representative for many relict species and populations. Thus, this compilation is more than a simple monography on the Violet Copper. Authors from all over Europe describe the history and ecology of remnant populations, raise the question on how and why to protect relict occurrences and highlight the relevance of combining different methods, which are described in six method boxes. Finally, this book also is a plea for bringing together theoretical and practical conservationists.



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